MORPHOLOGICAL STUDIES OF IRRADIATION EFFECT ON SOME CROP PLANTS

SUMMARY OF

THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BOTANY

S. A. Chaghtai

ALIGARH MUSLIM UNIVERSITY

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C E R T I F I C A T E

It is hereby certified that the thesis entitled "MORPHOLOGICAL STUDIES OF IRRADIATION EFFECT ON SOME CROP PLANTS" submitted by Mr. S.A. Chaghtai, Professor of Botany, Saifia College, Bhopal for the award of Ph.D. degree is a record of his independent, original investigation carried out under my guidance and supervision.

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CERTIFICATE

This is to certify that the thesis entitled "MORPHOLOGICAL STUDIES OF IRRADIATION EFFECT ON SOME CROP PLANTS" is a bonafide work carried on, by the candidate - Mr. S.A. Chaghtai under the supervision of undersigned. It is to be submitted for the award of the degree of Doctor of Philosophy in Botany.

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ACKNOWLEDGEMENT

I am highly indebted to Dr. S.C. Chakravarti, M.Sc., Ph.D., D.Sc., F.N.A.Sc., Retired Professor of Botany and ex-chairman, Board of Studies in Botany, Bhopal University, Bhopal and Mr. Zia Uddin Ahmed, Reader in Botany, Aligarh Muslim University, Aligarh for their valuable suggestions, guidance and supervision. I am equally indebted to Professor A.K.M. Ghouse, F.L.S. (Fellow of Linnean Society, London) of A.M.U., Aligarh who painstakingly helped me in the preparation of the manuscript.

I will be failing in my duties if I do not express my deep sense of gratitude towards my young colleague Mr. S.S. Khan for his assistance, inspiration and encouragement I perpetually received from him. He helped me in a number of ways till the submission of the thesis.

My thanks are also due to Professor S.K. Saxena, Head, Department of Botany, Aligarh Muslim University, Aligarh, Shri Fakhruddin, Secretary, Saifia Educational Society, Bhopal, Shri Shakoor Khan, Principal, Saifia College, Bhopal, who were so kind as to made me available the laboratory and library facilities under their respective control.

Lastly I am grateful to all those authors and research workers upon whose works I have freely drawn and whose names are beyond the scope of this brief acknowledgement.

These acknowledgements will be incomplete if I fail to thank Mr. P.M. George who gave the final shape to this thesis by way of typing.

26.10.84

(S.A. CHAGHTAI)

C O N T E N T S

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INTRODUCTION

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I N T R O D U C T I O N

Ever since the discovery of X-rays and radioactivity, the ability of the high energy particles to penetrate the living systems has excited a great and sustained interest in biologists. Earlier data which led to the formulation of quantitative hypotheses in radiobiology was principally concerned with such biological entities as bacteria, viruses and enzymatic systems which neither have a nervous system nor a complicated physiology. Few attempts have been made to use higher plants or animals for testing quantitative hypotheses, desirable though it would be on many grounds to obtain further insight into the action of radiation on higher organisms. Higher plants provide exceptionally favourable material for many basic studies in radiobiology. One aspect of the usefulness of plant systems involves the feasibility of experimentally separating growth, cell division and senescence etc. from one another. Plant development represents a regular pattern of growth differentiation processes including numerous correlative phenomena. Exposure of green plants to ionizing radiations may upset this balance and induce the morphological changes in cells. Such changes are usually reflected as morphogenetic abnormalities.

In recent years ionizing radiations have been greatly exploited in understanding various fundamental problems of life processes and in improving crops through mutations and subsequent breeding of these mutants. The ability of ionizing radiations to speed up the frequency of teratological changes has been utilized to throw light on the morphological nature of the organ. Radiation, therefore, may be considered as a valuable tool for developmental analysis, particularly where many events in growth are obscured (or not observed) because of the time factor.

A considerable amount of literature is presently available on the effects of ionizing radiations on plants (Johnson, 1936; Sparrow, 1951; Gunckel and Sparrow, 1954; Bacq and Alexander, 1961; Sparrow and Evans, 1961; Bari, 1971; Nayar, 1971; Sinha and Godward, 1972; Gupta, 1976; Nair and Nair, 1972; Chaghtai *et* al., 1978; Murty, 1979; Dwivedi and Pandey, 1982; Bandyapadhyay and Bose, 1979; Khan Irfan A, 1982; Chaghtai *et al*., 1983). As pointed out by Gunckel (1957, 1965) the response elicited depends upon the species, its age, physiological conditions, radiosensitivity, dose rate and various environmental conditions. Gunckel (1957) has emphatically suggested that the results from one species or variety should not be expected in different plants or even from different stages of development in the same plant.

Studies pertaining to the effect of radiations on cultivated plants have been mostly confined to barley, wheat, broad bean and peas. Most of the important oil-yielding~~s~~ and proteinaceous field crops which have not yet received as much investigating attention as they deserve are: Sunflower, Sesame, Soybean, Lentil, some Madhya Pradesh cultivars of flax etc.

Keeping in view the progress made in this field with other crop plants and the enormous scope of further research, a comparative study of Biological Effects of gamma irradiation on *Linum* an oil seed and *Lens* a proteinaceous plant was undertaken.

The present study was primarily aimed at:

1. gaining further insight into the phenomenon of irradiation efficiency.

2. estimation of the relative effectiveness of gamma rays in the two genera Gena upon qualitative and quantitative characters.

In these experiments seeds of *Linum usitatissimum* variety-NP (RR)5 and *Lens culinaris* variety-JLS-3 were subjected to six different doses of gamma rays. The sensitivity of gamma rays on these crop plants has been estimated through genotypic irradiations against varying doses in immediate generation while effectiveness and variability of quantitative characters is estimated in R_2 generation. The results obtained from all these studies are presented in this thesis.

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REVIEW OF LITERATURE

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REVIEW OF LITERATURE

A. GENERAL ACCOUNT

General morphological effects following irradiation have been described recently by many authors (Ehrenberg et al.,1953; D'Amato, 1957; Preadcencu Al et al.,1961; Bari, 1971) and have been reviewed earlier for acute X-irradiation (Johnson, 1936; Swaminathan and Kamara, 1961) and more recently for chronic gamma-irradiation (Sparrow, 1951; Gunckel and Sparrow, 1954; Gunckel, 1957; Beard, 1970 and 1971; Yadav and Dalal, 1971; Lobana et al.,1973; Abraham and Desai, 1976).

Sparrow and Evans (1961) have published bibliography on the effects of ionizing radiations on plants from 1896 through 1955. Bacq and Alexander (1961) have done commendable job in their books entitled "Fundamentals of Radiology" and "Cellular Radiology" respectively. Proceedings of series of a symposium on the effects of ionizing radiations on seeds, published by International Atomic Energy Agency, Vienna (1961), provides a good deal of information on the subject.

B. SEED GERMINATION

Effects of X-rays and gamma-rays on seed germination in higher plants have been studied by several workers such as Johnson (1928), May and Rosey (1958), Gustafson and Simak (9958), Bora (1961), Bowen and Thick (1961), Harring *et al.*, (1964), Rai (1971), Bari (1971), Ananthaswamy *et al.*,(1971), Chopra (1972), Ravindranath (1974), Chaghtai *et al.*, (1978 a, b, c), Abidi and Ghouse (1979), Chaghtai and Prasad (1979 a, b).

Rajan (1969) reported certain lower doses of ionizing radiations to be stimulatory for seed-germination in *Sesamum* while delayed germination has been reported in the gamma-irradiated dry seeds of *Corchorus* sp. and *Phaseolus vulgaris*. Bari (1971) found no clear difference in the germination of *Linum* seeds given acute doses from 10 KR to 250 KR of gamma rays.

Raghuvanshi and Singh (1977) while studying the effects of gamma-rays and some chemical mutagens on seed germination and seedling morphology of *Capsicum annuum* L. observed that both physical as chemical mutagens affected the percentage of seed-germination and a slight shift in most of the treatments but 0.03% DDA induced earliness. Gamma-rays alone or in combination with DMS or DMSO deferred in the day of initiation. The effect was found to be a synergistic one with gamma-rays + DMS. The effect of combination treatments on the frequency of germination was almost intermediate to that of individual mutagens. Maximum delay of 12-16 days in initiation of emergence was recorded with higher doses of gamma-rays (40 KR).

Recently Abidi and Ghouse (1979) while studying the effect of acute gamma-irradiation on the seed germination of *Linum usitatissimum* L. Var. *Neelam* observed that lower doses like 25 KR or 50 KR effectively promote the germination process to the extent of about 20% over the control when applied to the seeds in dry condition, while higher doses like 100, 125 or 150 KR bring about a gradual decline in the process in a positive manner. In case of gamma-irradiated seeds of *Capsicum annuum* Var. K_2, Chaghtai and Prasad (1979 a) observed an inverse linear correlation between the germination percentage and dose rate. Almost similar results were obtained with Var K_1 (Chaghtai and Prasad, 1979 b).

Caldecott (1954) described an inverse relationship between water content of seeds and their sensitivity to X-Rays. Later working with barley seeds, he found that sensitivity decreased as the water content increased from 4% to 8%. Further addition of water resulted in no additional modification of sensitivity. Gustafsson and Simak (1958) also found an inverse correlation between water content and X-ray sensitivity in pine seeds. Immature seeds were found more sensitive than fully matured ones. At equal doses, X-rays had a stronger depressive effect than gamma-rays. Chaghtai et al.,(1978 a, b) reported a notable difference in germination response of the dry and presoaked gamma-irradiated seeds of Phaseolus mungo and Lens esculenta. For P. mungo similar results were also reported by Siddiqui et al.,(1979).

Ehrenberg et al.,(1953) reported that a change in oxygen pressure at the time of irradiation has profound effect on physiological processes, behaviours and responses examined in barley seeds.

Once the germination process is initiated, radiation sensitivity is altered. Thus the response of germinating seeds as well as of young seedlings towards irradiation is markedly different as compared to the unactivated or dormant seeds. Nybom (1956) found that a number of seedlings reacted in the same manner whether the radiation was continuous or intermittent during 12 hrs. either day or night. On the other hand Biebl (1959) found a notably higher sensitivity as measured by the depression of subsequent organ growth when he X-rayed germinating wheat grains on the second day of germination (since the soaking of seeds).

It has been recognised that temperature, water content, oxygen tension, radio protective substances in the seed and the type of ionizing radiation may all affect seed-germination as well as growth of seedlings (Nybom et al., 1952; Caldecott, 1955 a,b,c; Conger and Randolph, 1959).

In addition to the factors listed above, radio-sensitivity of a plant has also been reported to depend on its Karyotype. Plants with comparatively smaller chromosomes tend to be less sensitive than those with larger ones. Within the same genus normal diploids have been found more sensitive towards radiations than the polyploid species (Sparrow and Evans, 1961).

Recently X-rays have been reported to stimulate germination of some highly dormant wild papilionaceous seeds (Chaghtai *et al.*, 1978 c).

C. SEEDLING SURVIVAL

Reduced seedling survival as an after effect of irradiation has been reported by Caldecott (1955 b) and Rai (1971). Bari (1971) also found that survival of flax plants was very low at 150 KR and 200 KR doses of gamma-rays and there was practically no survival at 250 KR.

D. GROWTH HABIT RESPONSES

Sparrow (1951) noticed an increase in plant height of *Antirrhinum majus* at exposure rates above 125 R/day, reaching to maximum at 230 R/day. Gunckel and Sparrow (1954) reported similar stimulation of growth and notable increase in plant height, in *Antirrhinum* and *Nicotiana* spp. when subjected to moderate exposure of chronic gamma-irradiation. Ehrenberg *et al*.,(1954) observed growth stimulation in *Vicia faba* at daily exposure range of 16-28 R of gamma-irradiation. Studying the effect of Chronic gamma-irradiation, Mikaelson and Aastveit (1957) observed a considerable increase in plant height at exposures ranging from 25 to 34 R/day in barley. Similar behaviour was recorded by Sparrow and Evans (1961). De Nettan Court and Contaat (1966) found marked increase in plant height of *Lycopersicum* sp. under continuous radiation exposures. Davies (1968) recorded growth stimulation due to irradiation in a wide variety of higher plants. Bostrack and Sparrow (1970) observed a significant increase in height at exposure rates of 1-2 R/day in *Pinus strobus*.

On the contrary there are equally numerous reports regarding the inhibition of seedling growth and height of plants under the influence of irradiation. Johnson (1936) held the opinion that injury and growth are the most common effects following X-ray exposure. Sparrow (1951) reported a general decrease in plant height with every increase in dose level. D'Amato (1957) in his studies on the effect of Chronic gamma irradiation in flax did not find any growth stimulation except in the early stages of plant growth at 375 and 625 r/day. It was, however, not ascertained as to whether the difference was due to some stimulatory action of radiation or to some differences in soil condition. Since then growth inhibition by ionizing radiations has been reported in various plants by many workers (Gunckel and Sparrow, 1961); Dumanovie and Ehrenberg, 1965; Davis, 1968; Chauhan, 1969; Rai, 1971 and Chopra, 1972). Bari (1971), during his studies on acute irradiation on flax, found that the plant height at maturity decreased with increasing exposures. While on the other hand, in the chronically exposed plants of flax he noticed that plant height at maturity increased gradually as the daily exposure rate increased from 100 to 600 R followed by a sharp decline thereafter. At 1000 R the plant height was less than that of the non irradiated plants. Maximum average height was observed in plants subjected to a daily exposure of 600 Rs

Different workers hold different opinion regarding the phenomenon of stunted growth resulting from irradiation. Important suggestions are: (1) uneven damage to meristematic cells due to genetic injuries (Gray and Scholes, 1951; Lea, 1955), (2) Chromosomal damages or inhibition of cell division (Sparrow and Evans, 1961; Conger and Stevenson, 1969), (3) marked decrease in the auxin level following irradiation (Moh and Smith, 1951; Gordon, 1954, 1957), (4) marked effect on auxin synthesis (Gunckel and Sparrow, 1961) and (5) effect on respiratory enzymes (Bjornseth <u>et al</u>., 1957).

Quastler <u>et al</u>, (1952) held both irradiation and physiological disorders responsible for the stunted growth.

E. STEM MODIFICATIONS

X-ray induced stem abnormalities were observed by Johnson (1936) while Gunckel *et al.*, (1953) reported similar abnormalities induced by gamma rays in case of *Tradescantia* where the young stem exhibited distinct swelling at the base of upper internodes accompanied with slight twisting of the branches but the effect was found temporary and short lived as the normal pattern was soon restored with the advancement of growth.

Common responses of stems to irradiations are dwarfing and excessive branching from abnormal development of axillary or adventitious buds. Irradiated stems may also show swelling and twisting of internodes, fasciations, dichotomy, altered phyllotaxy, stem lesions, an increase in the amount of vascular tissue and formation of stem tumours (Gunckel and Sparrow, 1954).

Fasciation and similar growth abnormalities in stem and leaves have been observed following irradiation with X-rays (Irvine, 1940) or gamma-rays (Gunckel and Sparrow, 1954; D'Amato, 1957). Ionizing radiations and growth regulators have been shown to bring about identical fasciation in plants (Gorter, 1965). In higher plants particularly the induction of fasciation following irradiation is of most frequent occurrence.

Induction of dichotomies and altered phyllotaxy have also been demonstrated in certain cases following exposure to gamma-rays and thermal neutrons (Johnson, 1933, 1948; Irvine, 1940; Gunckel, 1965).

Fasciation of flower stalks due to X-irradiation has been observed in sunflower (Johnson 1926) and flax (D'Amato, 1957). In irradiated and fasciated stems of _Linum_, D'Amato (1957) reported the xylem ring to be thinner than in control and there was greater variability of fibre-cell diameter and wall thickness although fasciation did not affect fibre length. Abidi _et al._ (1979 a) and Ghouse _et al._ (1980) while working on an Indian oil yielding variety T397 of flax observed that gamma-irradiation seriously affects the development of primary body as well as secondary structures in a direct correlation to the dose rate. The greater was the level of radiation intensity, the more was the damage caused to the water conducting system as well as to the mechanical tissues of the plant.

Sparrow (1951) and Sparrow and Evans (1961) reported development of adventitious meristems and tumours on the irradiated stems of Nicotiana glauca with higher dose rates or longer exposure at any one dose. The increase in number of plants with tumours was accompanied by an increase in the amount of tumour per plant.

F. LEAF ABNORMALITIES

Reduction of leaf blade, twisting of the entire leaf or leaflets and fusion of leaf parts have been reported as common features in irradiated Helianthus seedlings (Johnson, 1926) and tomato plants (Johnson, 1931). Similar results for a number of plants were reported by Goodspeed (1929), Morgon (1931), Haskins and Moore (1935). Leaf thickening has been shown to increase with dose rate (Gunckel and Sparrow, 1954). Response of the leaves of Antirrhinum majus was studied at 17 dosages of gamma-rays ranging from 0.5 to 600 R/day. A progressive thickening of the leaves was observed above 240 R dose which increased to three times that of controls at 600 R/day and the leaves had a leathery texture. Beibl (1959) also observed similar effects of gamma-irradation on leaves of higher plants.

Abnormalities pertaining to leaf, colour, form and texture have been found to be induced by several workers in many plants following suitable doses of ionizing radiations. Leaves show a wide range of responses like the appearance of a mosaic or over all colour changes, dwarfing, premature abscission, increased pubescence, changes in leaf form and texture accompanied with irregular blade development such as distorted venation, puckerring between the veins, fusions, thickening, development of double leaf-blades and formation of tumours (Johnson, 1936; Gunckel and Sparrow, 1961; Gunckel, 1965; Bajaj et al., 1970; Chopra, 1972; Seetharam and Srinivasachar, 1972; Bandyopadhyay and Bose, 1979).

G. FLORAL TERATOLOGY

In addition to stimulation of growth in length of floral stalks and abnormal thickening including fasciation, irradiated plants have been found to show: (1) abnormal vegetative growth in floral positions (Sparrow, 1951; Gunckel, et al., 1953 a, b), (2) delayed or reduced flowering, (3) colour changes and other somatic mutations, (4) high degree of sterility, (5) early abscission, (6) modification in form and number of floral parts especially those of petals and stamens (Gunckel et al., 1953 b). Most of these effects are discussed in detail in a review paper by Gunckel and Sparrow (1954).

Irradiated sunflower plants have been reported to produce fasciated flowers (Johnson, 1926) while production of multiple flowers or abscission of flower buds (depending upon the stage of development at the time of irradiation) have been observed in case of tomato (Johnson 1931). Haskins and Moore (1935) reported premature flowering in grape fruit plants from X-irradiated seeds. Johnson (1936) reported delayed and reduced flowering in a number of plants. Early blooming in a group of irradiated *Kalanchoe* plants has also been observed (Johnson, 1948). The inflorescence of *Tradescantia paludosa* receiving 20-24 R dose/day for 8 weeks proliferated into a globose head by the formation of leaf-like structures and modified flowers. Removed from radiation source and allowed a recovery period, these heads developed a large number of apparently normal vegetative shoots (Gunckel *et al.*, 1953 a). Abscission of flower buds in tomato, tobacco, snapdragon and several other plants growing in gamma-field has been reported by Gunckel and Sparrow (1954). Stimulated flowering has been demonstrated in *Tradescantia paludosa* (Gunckel *et al.*, 1953 b), *Nicotiana rustica* (Gunckel and Sparrow, 1954), *Impatiens sultanii* (Gunckel, 1957). It has been found that with the increasing doses of chronic gamma rays flowering is generally retarded (Gunckel, 1965). Bari (1971) reported delayed flowering in *Linum* with prolonged radiation exposure and at 100 R/day flowering was initiated about a month later as compared to the control.

Occurrence of colour chimeras in the flower after X-ray treatment was observed by Moore and Huskins (1935). Appearance of similar chimeras has been reported in several plants by Sparrow (1951).

Johnson (1936) observed sterility to be a common by-product of X-ray treatment. Sterility was quite common at high dosages in chronically irradiated plants, particularly in those flowers which were premature in flowering (Gunckel et al., 1953 a; Gunckel and Sparrow, 1954). These findings have been reaffirmed by Kumar (1971), Kumar and Singh (1972), Rogers and Xavier (1972). In Linum usitatissinum L. Var. T397, Abidi et al., (1979 b) found that gamma rays induced male sterility in a direct correlation to the dose rate. The progeny turned out to be almost completely male sterile at 150 K rad while it was 50% at 25 K rad dose.

The most frequent modifications in floral development after irradiation are in the form and number of flower parts. Abnormalities commonly include modified petal lobes, increase or decrease in the number and size of petals or inhibition (Johnson, 1936; Gunckel *et al*., 1953 a; Horland *et al*., 1973).

Bordy (1953) showed a wide range of abnormalities in tomato including multiple, fasciated or single, open or closed ovaries, multiple or fused styles, extra stamen traces, formation of meristematic areas on anther or filament which gave rise to embryo sacs and microspore as well as megaspore mother cells in adjacent locules or even in the same locule of an anther.

H-Seed yield

Bari (1971) and Badwal *et al*.,(1972) reported abnormalities pertaining to seed-yield and seed-morphology in irradiated progeny of flax. Ghouse and Abidi (1979) while studying the effect of different acute doses of gamma-rays on the morphological and quantitative characters such as height and weight of the plants, number of capsules per plant etc. in Var. *Neelam* of *Linum usitatissinum* L observed no marked difference in the height and weight of the plants with 25 and 50 K rad doses which were at par with the controls but with 75 K rad dose they recorded a sudden rise in the number of capsules per plant. Doses higher than 75 K rad proved to be inimical or detrimental for height, weight and number of capsules per plant. Reduction in height with 100, 125 and 150 K rads was 16.9, 18.3 and 23.9 per cent respectively.

Mutation frequency in R_2 generation

It has been reported that higher frequencies of chlorophyll and other viable mutations are obtained with gamma irradiation and other physical mutagens (Blixt et al., 1958 and 1963; Zannone, 1965). Sharma (1965) compared the effect of physical and chemical mutagens on Pea. Mugnozza (1966) observed in *Triticum durum* that physical mutagens are more effective than chemical mutagens. Monti (1968) obtained very high frequency of chlorophyll and viable mutations with treatments of DES and X-rays. Jacob (1970) studied the comparative mutagenic effect of chemical mutagens and gamma rays. Swaminathan et al.,(1971) observed frequency and spectrum induced in rice varieties by physical and chemical mutagens. Hussain *et al*., (1974) studied chlorophyll mutations in *Pisum* by EMS and gamma rays. Meono (1977) studied chlorophyll mutation by gamma-rays in *Phaseolus*.

Effectiveness and efficiency

Monti (1968) reported that in pea the effectiveness of DES was 3 to 4 times more than gamma rays. Hussain (1969) also calculated the efficiency of EMS and X-rays in *Arabidopsis thaliana*. Swaminathan *et al*.,(1971) studied the efficiency of physical and chemical mutagens in dehusked rice in different pressure conditions. Race (1977) also compared the effectiveness and efficiency of EMS and gamma-rays in rice. Sinha and Bose (1978) observed that chemicals are more potent to induce the effectiveness and efficiency in rice.

Macromutations

Gaul (1961) defined two categories of mutations (i) macromutations, (ii) micromutations. Macromutation involve gross changes in phenotypes which can be recognised with certainty in a single plant. Scholz (1967) successfully developed in a single variety of barley nearly as wide a variability as found in the world collection of germplasm material through macromutational selection.

X-ray induced macromutations in *Sesamum* have been reported by Rai and Jacob (1958), Nayar (1969), Swant and Dhagat (1970) and Nayar (1970). Sharma and Sharma (1979) reported leaf mutations induced by gamma-rays and NMU in Lentil.

Micromutations

The mutational changes which can be isolated and fixed,only through adapties of biochemical procedure are called micromutations (Swaminathan, 1964).

Extensive work was done by Gregory (1955, 1956, 1957 and 1959) on micromutations of groundnut. His pioneer work was very important in radiation research as applied to plant breeding. The results obtained by Gregory showed that mutations affecting a quantitative character in a crop plant can be induced by radiation and that phenotypic selection can accumulate positive mutations to produce better mutants. Bansal (1969) studied the polygenic mutations in barley. Mandal (1974) studied the quantitative characters in gram. In groundnut, Sharma (1975) observed significant increase in overall variance for different macro characters. Nayakar (1976) reported genetic variability heretibility and genetic advancement for six quantitative characters. In _Sesamun_, Swant (1971) and Dixit (1975) induced quantitative variability for different characters by irradiation.

MATERIAL AND METHODS

MATERIAL AND METHODS

MATERIALS

Two crop plants *Lens culinaris* variety - JLS-3 and *Linum usitattissemum* variety NP (RR)-5 which are common cultivars in Madhya Pradesh were selected for studying their behaviour towards gamma irradiation. Certified seeds of both these species were procured from J.N.K.V.V., Jabalpur. Healthy and undamaged seeds of similar size were handpicked for experimental studies.

METHODOLOGY

Method of gamma irradiation

Fully mature and healthy seeds of uniform size, free from mould and mechanical injury were selected for treatment. To determine the effective range of gamma rays pilot experiments were conducted in the preceding year with the two genera by way of employing wide dose range. Period of presoaking the seeds making them vulnerable to the action of different mutagens was also ascertained through preliminary experiments.

Gamma ray treatment

Seeds presoaked in distilled water for 12 hours arranged in monolayers were subjected to six different acute doses of gamma rays from Co^{60} source emiting from a portable unit of "Pantatron" (AEI-England) at the strength of 2.5 U, of 5, 10, 15, 20, 25, 30 Kr from B.H.E.L., Bhopal Unit. A set of untreated seeds with same moisture content was used as control. For each treatment 4 replicates each of 100 seeds were used.

Method of handling the treated material and recording observation pertaining to R_1 and R_2 generations

A. R_1 generation

(i) Raising of irradiated progeny

The seeds treated with different doses of gamma rays, alongwith their control were sown in lines in three replications with one hundred seeds each. The plant to plant and line to line distance was 6" x 6", one set with each treatment and one of untreated material were kept for germination studies in petri-dishes containing moist filter paper pads. About 40 to 50, R_1 plants were selfed by way of covering the axils of fertile branches with thin polythene bags just before flowering. The bags were removed after the first flush of flowering was over.

(ii) Methods of recording observations pertaining to R_1 generation

Sensitivity of the two genera towards different doses of gamma rays was studied with the help of following parameters.

(a) Seed germination

Since germination tends to be delayed in the treated material, observations on germination of treated seeds and their controls were recorded daily for 20 days after sowing, to study and ascertain the period of delay caused due to the toxic effect of gamma rays. Finally the overall germination percentage was calculated. The emergence of the tip of radicle was taken as the indication of seed germination.

(b) Growth rate

Effect of different irradiation doses on growth rate was determined in terms of root and shoot elongation. For this purpose mean of the data collected for 40 seedling was taken. The values of growth in length have been expressed in centimetres.

(c) Survival of seedlings

The survival percentage was computed on the basis of values obtained with respect to percentage of plants surviving till maturity, out of the total number of plants, produced through seed germination.

(d) Chimeras and other morphological abnormalities

In the R_1 progenies appearance of chlorophyll chimeras and other morphological abnormalities were noticed, which were carefully recorded and thereafter their frequencies of occurrence were calculated.

(e) Pollen sterility

Pollen sterility was determined from mature anthers of 25 randomly selected plants for each dose by way of staining the pollen with 2% acetocarmine mixed with equal quantity of glycerine in both the plants. Pollen grains which failed to take stain or exhibited abnormal shape accompanied with improper filling were considered sterile. Percentage of occurrence of such sterile pollen grains was calculated.

(f) Seed sterility

Ten fruits from each of the 25 randomly selected fully mature R_1 plants per treatment were studied for sterility. From each pod in *Lens* and capsule in *Linum* thin and papery seeds were sorted out which were taken as sterile and their percentage of occurrence for each pod or capsule was determined with the help of which average seed sterility of 25 randomly selected plants was determined and it was regarded as sterility due to irradiation.

B. R_2 generation

(i) Raising of R_2 generation

R_2 generations were raised on the experimental plots. In all the treatments, involving both the plants, genetic variability was studied in comparison with their controls. For this purpose 60-75 seeds obtained from 25 normal looking R_1 plants for each treatment were grown in randomised plots. Progeny of each R_1 plant was replicated thrice and 20 or 25 seeds per replication were sown. These seeds were sown in 1 metre long rows. Distance between plants was 15 cm while it was 30 cm between the two rows.

In order to study the frequency and spectrum of macromutations, remaining seeds of R_1 progenies of both genera were grown separately as progeny rows. The crop was irrigated twice and other cultural and agronomic practices were followed in the same way as carried out for raising R_1 generation.

(ii) Method of recording observations

(a) Chlorophyll mutations

The population was carefully screened for chlorophyll mutations during the first month after seed germination in both the plants. The nomenclature of chlorophyll mutations and classification procedure proposed by Gustafsson (1947) was followed which are detailed in table-1. The following methods were adapted to calculate the chlorophyll mutation frequency.

1) <u>R_2 family basis (% of mutated progenies in R_2 generation)</u>

It was calculated with the help of following formula.

$$\text{Mutation frequency} = \frac{\text{Number of mutated progenies}}{\text{Total No. of } R_2 \text{ progenies}} \times 100$$

TABLE - 1

Classification of various types of chlorophyll mutations with their conventional notations and definitions according to Gustafsson (1947)

Type	Notation	Definition
Albina	ALB	White seedling, neither chlorophyll nor carotenoids are present: Non viable.
Chlorina	CHL	Yellowish green colour, tending to become normal green, viable seedlings.
Xantho-alba	XA	Leaf base white with irregularly distributed yellow pigments towards the tip.
Viridis	VIR	Light green to yellow green semi viable.

2) <u>R_2 population basis (% mutants)</u>

The experimental values obtained were substituted in the following formula for determining the R_2 mutation frequency.

$$\text{Mutation frequency} = \frac{\text{Number of mutated plants}}{\text{Total number of } R_2 \text{ plants}} \times 100$$

(b) <u>Viable mutations</u>

Every R_2 progeny was examined repeatedly during the entire growth period for viable mutations affecting various morphological attributes. The inheritance pattern of some mutants was studied on the basis of R_2 segregation behaviour. The mutation rate was calculated on the basis of R_2 population and families as revealed through chlorophyll deficiency patterns.

(c) <u>Mutagenic effectiveness and efficiency</u>

The mutation rates (R_2 family and population basis) were made the basis for determining the mutagenic effectiveness and efficiency with the help of following formulae suggested by Konzak <u>et al</u>.,(1965).

Effectiveness of physical mutagen

The effectiveness of gamma rays was determined with the help of following formula.

$$\text{Effectiveness} = \frac{\text{Mutation rate (}M_2\text{ family or population basis)}}{\text{Dose in kilo reentgens}}$$

Efficiency of the gamma rays used, was determined by way of substituting the experimental values in the following formula.

$$\text{Efficiency of irradiation} = \frac{\text{Mutation rate (}M_2\text{ family or population basis)}}{\text{Percentage of lethality}}$$

Micromutations

Observations on the induced genetic variability for five quantitative characters of economic value were computed for both the genera. For each replication, data for five normal looking plants were recorded. Thus, 10 plants per R_2 family and 500 plants per treatment were screened. Following five characters were selected for study.

1) Days to flowering

It is defined as the total time recorded in terms of days taken by a plant from sowing to the opening of first flower.

2) Number of branches per plant

The number of branches per plant were recorded in the field when the plants were fully mature.

3) Number of fruits per plant

At the time of maturity in both genera, from 25, randomly selected plants from each treatment as well as control were studied for comparison.

4) Weight of fully mature 1000 seeds

Random samples of 1000 seeds were collected from the produce of each plant and the weight of these seeds was recorded in milligrams.

5) Seed yield per plant

At the time of maturity the pods and capsules of each plant were picked up one by one and packed in separate polythene bags, thrashed separately and thereafter the seeds obtained from each plant were separately weighed and their average was calculated to determine the seed yield per plant in both genera.

D. Statistical methods

(i) R_1 generation

Data collected on various characters in R_1 generation were analysed according to randomized plot design as worked out by Panse and Sukhatme (1967), after calculating the block means. Based on critical difference (C.D.) and standard error (S.E.) the significance or otherwise of the treatment effects was worked out.

(ii) R_2 generations

Data collected for five quantitative characters of R_2 generation was analysed. The details of the statistical analysis are given below:

a) Analysis of induced variability

Let the variate values of character be represented by x1, x2, x3 ------ xn, where n is the number of values. The various estimates of mean overall variance and co-efficient of variation were calculated as under:

1) Mean $(\bar{x})$ = $\dfrac{\sum_{i=1}^{n} x_i}{n}$

2) Over all variance (S^2) = $\dfrac{i}{n-1}\left[\sum_{i=1}^{n}(x_i)^2 - \dfrac{\left(\sum_{i=1}^{n} x_i\right)^2}{n}\right]$

i being the group interval.

(b) Analysis of components of variation and genetic parameters

For each treatment, analysis of variance was obtained for five characters, viz. days of flowering, number of branches per plant, number of fruits per plant, weight of 1000 seeds and seed~~s~~ yield on the pattern shown below:

Analysis of variance

Source	D.F (Degree of Freedom)	S.S (Sum of squares)	M.S (Mean square)
Replication	2		
Families			R_1
Error			R_2
Total			

In each treatment, based upon the families and error variance, the genotypic and phenotypic variance have been computed with the help of following formula:

Genotypic variance (VG) = $\frac{M1-M2}{r}$ where M1 is mean sum of square due to families and M2 is mean sum of square due to error, r being the number of replicates.

Phenotypic variance (VP = VG + M2)

The phenotypic variance (VP) and genotypic variance (VG) were further used to compute components of variation and genetic parameters.

a) Components of variation

(i) Phenotypic coefficient of variation

(PCV) = $\frac{\sqrt{VP}}{\bar{x}}$ X 100 , where x is the mean of values.

(ii) Genotypic coefficient of variation

(GCV) = $\frac{\sqrt{VG}}{\bar{x}}$ X 100 , where x is the mean of values.

b) <u>Genetic parameters</u>

(i) <u>Heritability (h^2)</u>

Heritability was estimated as a ratio of genotypic variance to phenotypic variance using the following formula:

$$h^2 = \frac{VG}{VP} \times 100$$

(ii) Genetic advancement was determined as percent of mean (GA).

The formula used was:

$$GA = \frac{\sqrt{VP} \times h^2 \times K}{\bar{x}}$$

<u>Abbreviations used</u>

C.D.	=	Critical difference
D.F.	=	Degree of freedom
G.A.	=	Genetic advancement as percent of mean
G.C.V.	=	Genotypic coefficient of variation.
h^2	=	Heritability.
i	=	Group interval the value of which is given at sequence.
K	=	Constant, whose value at 5% selection intensity is 2.06.
Kr	=	Kilo roentgens.
M1	=	Mean sum of square due to families
M2	=	Mean sum of square due to error
R_1 generation	=	Immediate generation following gamma ray treatment.
R_2 generation	=	Progeny of R_1 plant seeds.
M.S.	=	Mean square
n	=	Number of plants
PCV	=	Phenotypic coefficient of variation
r	=	Number of replications.
s^2	=	Overall variance.
S.S.	=	Sum of squares
S.E.	=	Standard error
V.G.	=	Genotypic variance
V.P.	=	Phenotypic variance
$\bar{x}$	=	Mean

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OBSERVATIONS

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OBSERVATIONS

Results obtained in the experiments of the present investigations are presented generation-wise below:

Observation on R_1 generation

The immediate effect of gamma irradiation was measured by seed germination, root and shoot elongation, survival of seedlings, chlorophyll deficient chimeras and morphological abnormalities. Pollen fertility of the plants emerging from the irradiated seeds was studied in *Lens culinaris* and *Linum usitattissimum* varieties JLS-3 and NP (RR)-5 respectively.

Seed germination

In gamma-irradiated seeds of both *Lens* and *Linum* there was decrease in germination percentage with increasing dose rate. Germination was above 95% in the controls of both the plants. Effect of gamma-rays was more pronounced on the germination percentage of *Lens* than of *Linum*. With 30 Kr dose

19.5% seeds of *Lens* germinated against 65.0% of *Linum* (Tables 2 and 3). LD-50 doses for both the plants are presented in Table 4. It can be seen from the table that the LD-50 dose of gamma rays for *Lens* is 10-15 Kr. While it is 30 Kr for *Linum*.

Root length

The results of root length indicate that in case of *Lens* the LD-50 varies from 15 to 20 Kr while in *Linum* it is 25 to 30 Kr (Table-4).

Shoot length

Like root length, as well as the mean shoot length also decreased with the increasing dose of gamma rays. However reduction in shoot length was less than in root at all levels of gamma irradiation in both the plants. The maximum percentage of reduction in shoot length was caused by 30 Kr dose in both (61.9% in *Lens* and 30.4% in *Linum*). Percentage reduction in shoot length was higher in *Lens* than in *Linum* (Tables 2 and 3). The LD-50 dose with respect to this parameter in both genera are presented in Table 4. It can be seen from the table that LD-50 dose for *Lens* was 20 Kr, while for *Linum* it was 30 Kr.

TABLE - 2

Effect of different doses of gamma-rays on germination, root and shoot length, plant survival, frequency of Chlorophyll deficiency, morphological abnormalities and pollen sterility in *Lens culinaris*, var JLS-3

Treatment & dose	Germination %	Root length Mean (cm)	Root length % reduction	Shoot length Mean (cm)	Shoot length % reduction	Plant survival %	Chlo. deficiency and morphological abnormalities %	Pollen sterility %
Control	97.5	7.2	0.0	14.2	0.0	100.0	0.0	0.0
Gamma rays (5 Kr)	72.0	5.3	22.2	11.3	4.9	71.5	5.8	4.0
" 10 Kr	63.5	4.8	28.1	9.2	12.0	58.0	15.3	12.0
" 15 Kr	42.0	3.7	38.5	7.4	20.1	40.5	18.4	14.0
" 20 Kr	28.0	3.2	62.0	6.9	32.0	38.0	27.6	22.0
" 25 Kr	21.0	2.8	73.0	6.2	59.0	35.0	36.2	32.0
" 30 Kr	19.5	2.2	80.1	5.1	61.9	29.1	48.3	36.0

TABLE - 3

Effect of different doses of gamma-rays on germination, root and shoot length, plant survival, frequency of chlorophyll deficiency, morphological abnormalities and pollen sterility in *Linum usitattissimum* var. NP (RR)-5

Treatment	Dose	Germi-nation %	Root length		Shoot length		Plant survi-val %	Chlo-defi-ciency & morphological abnormalities %	Pollen sterility %
			Mean (cm)	% redu-ction	Mean (cm)	% redu-ction			
Control	-	100	8.8	0.0	9.2	0.0	100.0	0.0	0.0
Gamma rays	5 Kr	100	7.9	4.9	8.9	3.7	98.0	0.0	0.0
"	10 Kr	100	6.3	12.3	6.8	5.9	82.0	0.0	2.0
"	15 Kr	92.0	5.6	21.7	6.7	10.1	80.0	14.3	6.0
"	20 Kr	87.5	4.9	32.0	6.0	14.2	72.0	17.2	9.7
"	25 Kr	72.0	4.0	40.9	5.4	22.3	67.0	22.4	13.2
"	30 Kr	65.0	3.2	59.8	5.2	30.4	58.0	35.7	22.7

Lens being proteinaceous is more radiosensitive than *Linum* which is oily.

Survival

The survival percentage ~~was~~ also show~~n~~ed a decrease with the increase in dose. In 30 Kr survival was 29.1% in *Lens* and 58.0% in case of *Linum*. The results clearly show that the reduction in survival percentage was higher in *Lens* compared to *Linum*. LD-50 doses of gamma rays for *Lens* ranged from 10 to 15 Kr and for *Linum* it was 25 to 30 Kr. Gamma ray sensitivity was found to be higher for *Lens* than for *Linum* under all doses (Table 4).

CHLOROPHYLL DEFICIENT CHIMERAS AND MORPHOLOGICAL ABNORMALITIES

It can be seen from tables 2 and 3 that chlorophyll deficiency and morphological abnormalities were induced under all doses in *Lentil*, while these abnormalities could only be produced with higher (15, 20, 25, 30 Kr) doses in *Linum*. In both the abnormalities showed an increase with the increase of dose. The percentage of these abnormalitiew was higher in *Lentil* than in *Linum*.

TABLE - 4

LD-50 dose of *Lens* and *Linum* varieties on the basis of different biological parameters.

Biological parameter	*Lens culinaris* variety JLS-3	*Linum usitattissimum* variety NP (RR)-5
	Gamma rays LD-50	Dose in Kr
Germination	10-15	30
Root length	15-20	25-30
Shoot length	20-25	30
Plant survival	10-15	25-30
Chlo-deficiency and morphological abnormalities.	30	30
Pollen sterility	30	

LD-50 dose for Lentil was found to be 30 Kr while that for Linum was more than 30 Kr. The selected crops showed a difference in respect to this parameter as in others (Table 4).

Pollen fertility

The control population produced fully fertile pollen grains (100% fertility), but the fertility fell with the increase of gamma-ray dose. The maximum pollen sterility in Lens was 36.0% with 30 Kr and in Linum 22.7% also with 30 Kr (Tables 2 and 3).

Since none of the doses could produce more than 36.0% fertile pollen in both the plants, the relative response of both the plants was determined at LD-25. Higher doses in comparison of 30 Kr are required (Table 4).

Here again Lens was found more sensitive to gamma-rays than Linum.

Radiosensitivity of *Lens* and *Linum*

Of the two plants, *Linum* has been proved to be more radioresistant than Lens.

Variety JLS-3 of *Lens* is highly radiosensitive for all the parameters studied, while *Linum* variety NP(RR)-5 is less sensitive in respect of all parameters studied and further higher doses cause more pronounced effect than lower doses.

Observations on R_2 generation

Dwarf plants

The effect of gamma-rays on plant higher was noted in all genotypes. They can be classified into three groups depending upon the level of reduction in height.

(a) Semi dwarf :

Plant grew to a height of 15-30 cm

(b) Dwarf :

Plant height - 10-15 cm

(c) Extreme Dwarf :

Plant height - less than 10 cm

TABLE - 5

Mutant types of *Lens culinaris* var. JLS-3 and their characteristics

Character	Control	Semi dwarf	Dwarf	Extreme dwarf
Average height (cm)	36.8	30.2	14.8	5.9
Average length of internodes (cm)	3.2	2.9	2.2	1.6
Average number of branches	6.3	5.0	4.8	2.9
Pollen fertility %	100	80-100	69-80	56-96
Average number of fruits.	126.5	90.5	52.2	14.9
Average number of seeds/fruit	1.7	1.8	1.4	1.2
Average seed yield per plant (gm)	3.8	3.4	1.6	0.5
Average 1000 seeds weight (gm)	20.8	20.7	20.4	20.0

TABLE - 6

Mutant types of *Linum usitattissimum* var.NP(RR)5 and their characteristics

Character	Control	Semi dwarf	Dwarf	Extreme Dwarf
Average height (cm)	33.02	14.12	10.12	3.92
Average length of middle internode (cm)	1.82	1.32	1.21	0.91
Average number of branches	-	3.0	2.1	1.9
Pollen fertility %	98	70-90	69-90	62-73
Average number of fruits	4.82	3.72	3.21	2.92
Average number of seed/fruit	26	24	-	20
Average seed yield per plant (gm)	8.7	7.34	6.1	5.9
Average 1000 seeds weight (gm)	14.29	13.97	12.23	10.9

Induced dwarfness was also found to be accompanied with other notable morphological features in _Lens_ and _Linum_ (Tables 5 and 6). It is clear from the table that the reduction in plant height was followed by reduction in all characters (Plate I, Fig. 3,4 & 5).

2) _Bushy habit_

In _Lens_ variety JLS-3, two bushy abnormal plants were observed. They were dwarf and bushy.

Bushy 1 (Fig. 10 & 11, Plate III)

These bushy plants were produced with the administration of 15 and 20 Kr doses of gamma rays. In R_2 generation segregation of these abnormal plants to normal as well as abnormal plants took place. In these plants fruits were of elongated type in comparison to normal fruit type of the control plants (Tables 7 and 8).

Bushy 2 (Plate III, Figs. 12 and 13)

The variants produced by the administration of 30 Kr dose of gamma rays were also of bushy habit. These plants also segregated in R_2 generation, their fruits were of very small size in comparison to normal

TABLE - 7

Bushy mutants of *Lens culinaris* var. JLS-3 and and their characteristics

Character	Control	Bushy-1	Bushy-2
Plant height (cm)	36.8	21.9	17.9
Colour of leaves	Green	Light green	Dark green
Days to flowering	103	119	111
Flower	Normal	Small	Small
Average number of branches/plant	6.3	3.4	3.7
Fruit size (mm)	9.0 X 5.2	9.2 X 5.1	9.0 X 3.9
Number of fruit/ plant	126.5	62.7	42.0
Seed yield/ plant (gm)	3.8	2.1	1.0
100 seed weight (gm)	20.8	20.9	19.8

TABLE - 8

Bushy mutants of *Linum usitattissimum* var. NP(RR)-5 and their characteristics

Character	Control	Bushy-1	Bushy-2
Plant height (cm)	33.02	30.12	-
Colour of leaves	Green	Dark green	-
Days to flowering	109	111	-
Flower	Normal	Normal	-
Average number of branches/plant	-	2.1	-
Fruit size (mm)	8.0 X 3.1	8.1 X 2.9	-
Number of fruits/plant	4.82	4.79	-
Seed yield/plant (gm)	8.7	7.9	-
1000 seed weight (gm)	14.29	14.27	-

PLATE - I

Fig. 1. A control plant of *Lens culinaris* variety JLS-3.

Fig. 2. Control plants of *Linum usitattissimum* variety NP(RR)5.

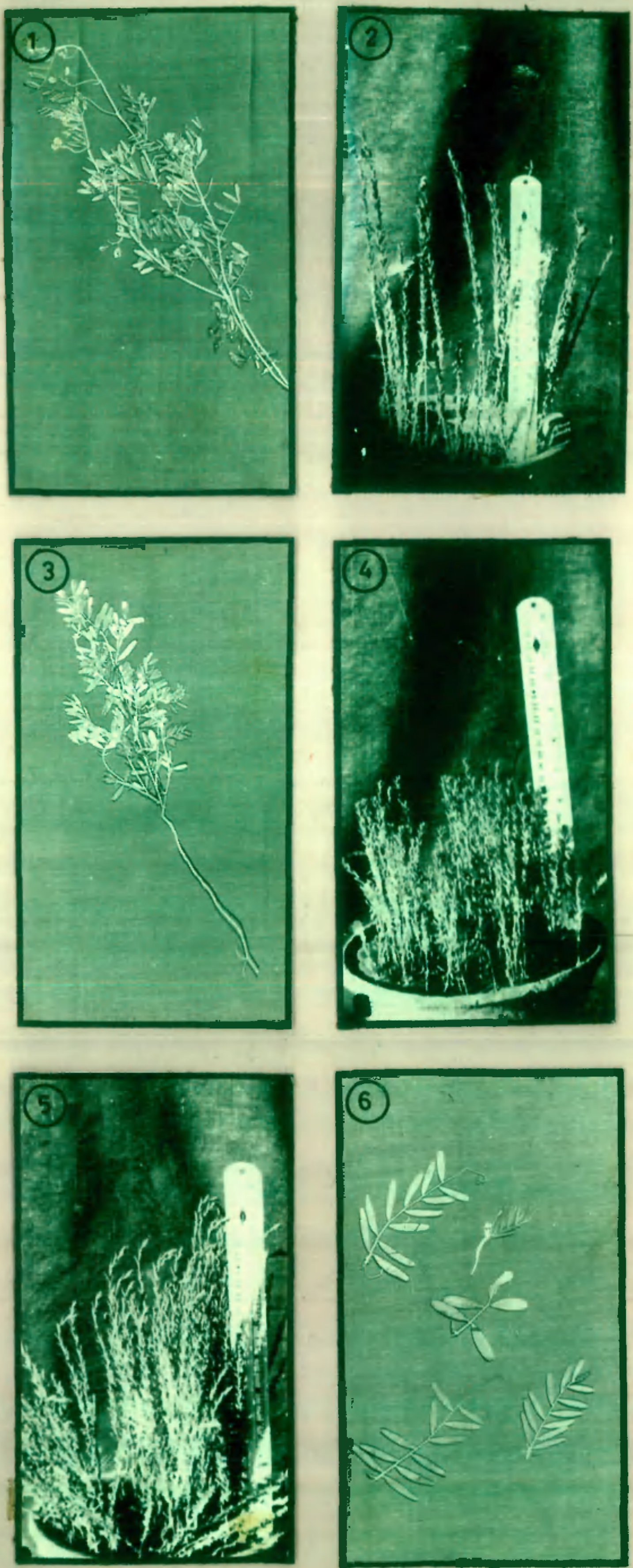
Fig. 3. A Dwarf mutant plant of *Lens culinaris* variety JLS-3.

Fig. 4. Dwarf mutants plants of *Linum usitattissimum* variety NP(RR)5.

Fig. 5. Semi Dwarf mutant plants of *Linum usitattissimum* variety NP(RR)5.

Fig. 6. Leaf size variations, with tendril formation in *Lens culinaris* variety JLS-3

PLATE 1



plants. The distinguishable characters of these two mutants are compared in table 5. In case of Linum 30 Kr dose of gamma rays yielded the abnormal plants which were highly bushy but belonging to one phenotype. These plants were of semi-dwarf nature in comparison to control. Their distinguishable characters are given in table 4 along with control (Tables 7 and 8).

3) Variation in leaf size

Lens normally possesses small leaves, but in gamma irradiated progeny variants were isolated, on leaf size basis. Characters of these leaf mutants are given in table 9.

In Linum, variation in leaf size followed a narrow range compared to Lens. Characteristics of variants are given in table 10.

In order to determine the variation in leaf size, 1000 seed weight correlation was calculated. The value ~~of~~ the correlation coefficient was 0.406 in Lens and 0.778 in Linum. These values of correlation co-efficient, were insignificant and suggestive of the fact that the two characters do not always go together.

TABLE - 9

Leaf mutants of *Lens culinaris* var. JLS-3 and their characteristics

Character	Control	Large leaf	Narrow leaf	Small leaf	Rolled leaf
Length of leaf (cm)	4.3	4.6	3.8	3.4	3.0
Length of Rachis (cm)	3.9	3.8	3.2	2.6	1.6
Length of leaflet (cm)	1.3	1.7	1.4	0.7	1.4
Width of leaflet (cm)	0.32	0.35	0.12	0.10	0.2
Number of leaflets/leaf	13.2	13.6	12.8	12.0	9.0
Flower	Normal	Large	Small	Small	Small
Number of fruits/ plant	126.5	121.3	110.0	98.2	111.0
Number of seeds/fruit	1.7	1.6	1.3	1.4	1.4
1000 seed weight	20.8	20.9	19.3	20.4	20.6

TABLE - 10

Leaf mutants of *Linum usitattissimum* var. NP(RR)-5 and their characteristics

Character	Control	Large leaf	Narrow leaf	Small leaf	Rolled leaf
Length of leaf (cm)	3.02	3.9	3.1	2.9	3.0
Length of Rachis (cm)	1.2	1.3	1.1	1.1	0.9
Length of leaflet (cm)	-	-	-	-	-
Width of leaflet (cm)	-	-	-	-	-
Number of leaflets/leaf	-	-	-	-	-
Flower	Normal	Normal	Normal	Normal	Normal
Number of fruits/plant	4.82	6.37	4.12	4.23	4.21
Number of seeds/fruit	8.7	9.2	8.2	7.60	7.92
1000 seed weight	20.8	20.8	20.1	20.1	20.3

It is clear from the table 9 that in *Lens* increase or decrease in dimension of the leaflet caused a proportionate change in the length of the leaf as well as rachis.

There was no change in the average number of leaflets per leaf in *Lens*. But this was not accompanied with the changes in other characteristics such as days to flowering, size of flower, pod and seeds etc. in both *Lens* and *Linum* (Plate II, Figs. 6, 7, 8 and 9).

4) Tendril variants

Only one such variant was observed in *Lens* where leaflets were found modified into tendril-like structures (Plate II, Figs 6 and 9). But a series of modifications of leaflet to tendril were observed including some intermediate narrow leaf types. This character also did not find to show any direct correlation with other characters of the plant concerned.

5) Floral variants

In *Lens* two types of flowers (1) open and (2) closed developed and all the flower parts in both the types were modified.

PLATE - II

Fig. 7, 8 & 9. Leaf size variations in *Lens culinaris* variety JLS-3 with different higher doses of radiation (gamma rays)

PLATE II

In normal plants of Lens calyx consists of five sepals and they were gamosepalous as in control plants but in variants sepals were free and their number (polysepalous) and colour were also different. The sepals were bifurcated and of light yellow colour.

This variant was early in flowering as compared to control, finally pod was formed with shrivelled seeds.

In Linum, flowers remained polysepalous and there was only a slight change in colour, where the other characters remaining unaffected. The flowering period sand seed setting also remained normal. Pollen fertility in this variant was normal in Linum but 20% sterility was observed in Lens.

Pointed pod

This variant was observed in the R_2 generation of Lens. There was one variant and 21 normal plants in R_2 progeny. The plants were of reduced vigour but showed normal height. It possessed small flowers and abnormal pod structure. The width of the pod was 4.2 mm compared to 5.6 mm recorded in control. The length of pod remained unchanged. However, the distal end of the pod was found narrow and formed a coiled structure. A high pollen sterility (70 - 80% was observed in such plants.

In R_2 progeny of the above, only few pods developed from a single plant with shrivelled seeds.

<u>Induced variability of fruits and seeds in *Lens* and *Linum*</u>

Fruit variants were observed in both *Lens* and *Linum* with gamma irradiations. The following types of fruits were obtained.

(a) <u>Large fruits</u>

In the R_2 generation of *Lens* variants were obtained on which large size fruits developed (Table 11). Large size fruits were also observed in *Linum* but their frequency was less in comparison to *Lens* (Table 12) (Plate III, Fig. 15).

(b) <u>Long fruits</u>

This type of variant was only found in *Lens*, whereas in *Linum*, such a type of variant was not observed (Tables 11 and 12).

TABLE - 11

Fruit mutants of *Lens culinaris* var. JLS-3 and their characteristics

Characters	Control	Large	Long	Small	Narrow
Length of fruit (mm)	9.0	12.8	13.1	7.2	7.1
Breadth of fruit (mm)	5.2	6.7	5.2	4.3	3.2
Size of seed	Normal	Normal	Small	Small	Very small
1000 seed weight (mg)	20.8	20.9	21.2	17.2	14.3
Shape of seeds	Round flatt-end	Round shri-velled	Round flatt-end	Slightly round	Rounded shri-velled
Testa colour	Mottled	Highly mottled	Slightly mottled	Mottled	Slightly mottled

TABLE - 12

Fruit mutants of *Linum usitattissimum* var. NP(RR)-5 and their characteristics

Characters	Control	Large	Long	Small	Narrow
Length of fruit (mm)	8.0	9.8	-	7.1	-
Breadth of fruit (mm)	3.1	3.6	-	3.9	-
Size of seed	Normal	Normal	Small	Small	Small
1000 seed weight (mg)	20.7	-	-	-	-
Shape of seeds	Normal	-	Small	Small	Small
Testa colour	Dark	Dark	Highly dark	Light	Light

PLATE - III

Fig. 10. Bushy-I mutant of <u>Lens culinaris</u> variety JLS-3.

Fig. 11. Bushy-I mutant of <u>Linum usitattissimum</u> variety NP(RR) 5.

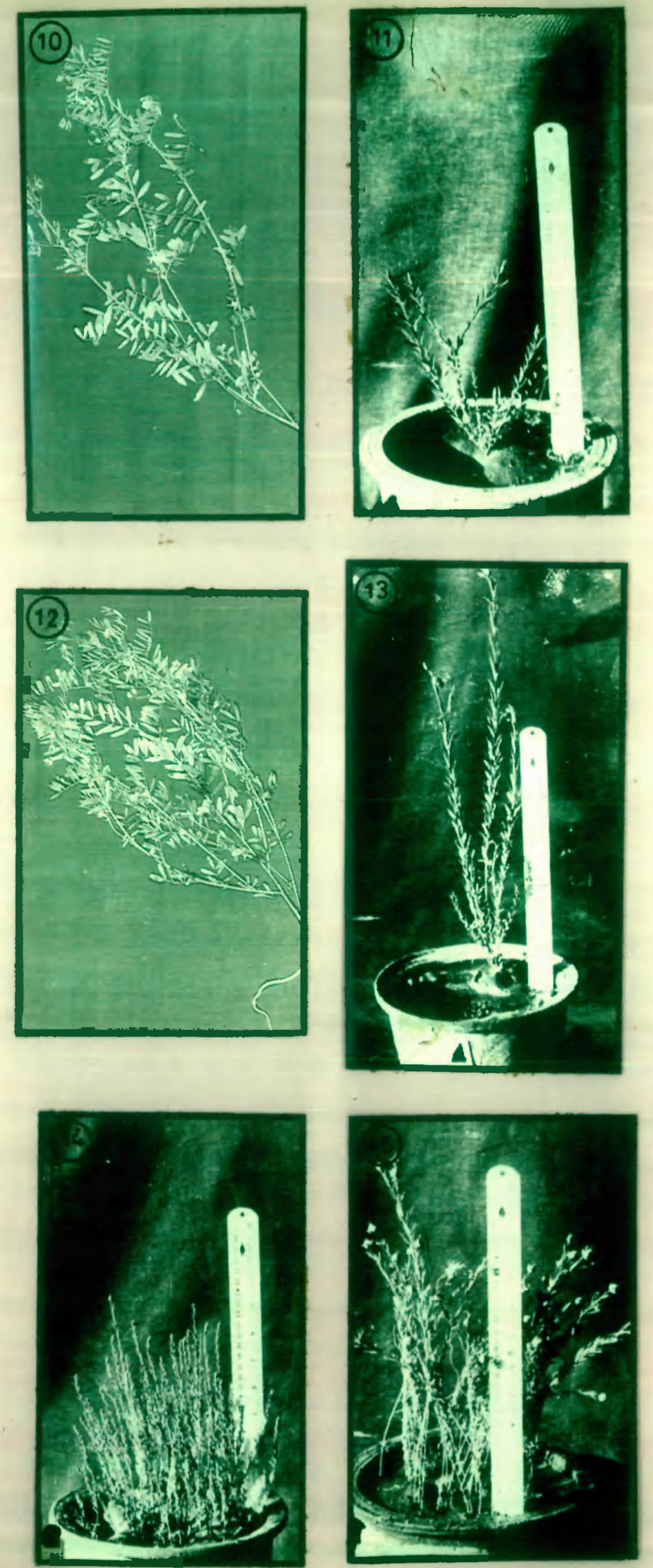
Fig. 12. Bushy-II mutant of <u>Lens culinaris</u> variety JLS-3.

Fig. 13. Bushy-II mutant of <u>Linum usitattissimum</u> variety NP(RR)-5.

Fig. 14. Small size fruit mutant of <u>Linum usitattissimum</u> variety NP(RR)-5.

Fig. 15. Large size fruit mutant of <u>Linum usitattissimum</u> variety NP(RR)-5.

PLATE III



(c) Small fruits

In both *Lens* and *Linum* small fruit variants were observed but the plants were dwarfs and they produced small flowers and small seeds (Plate III, Fig. 14).

(d) Narrow fruits

In *Lens* only this fruit variant was observed and fruit was found abnormally narrow and small though developed on normal plants (Table 11). It can be seen from the table that increase or decrease in size of fruits was accompanied by proportionate increase in the seed size. The correlation between surface area of fruit and 1000 seeds weight in *Lens*, was calculated and correlation coefficient was found to be 0.938 which is significant. In case of *Linum* the correlation coefficient was calculated between diameter of fruit and 1000 seed weight which was 0.898 and was also significant. Thus fruit size can serve as a convenient basis for selecting variability for seed size also (Table 12).

Chimeras for various other characters

After gamma-irradiation, both *Lens* and *Linum* produced tissues which were genetically different. This is a common feature in multicellular organisms where each cell produces a different variant independently. Such plants are called as chimeras. In the present study several chimeras were produced with gamma-irradiation which are as follows (Table 13 and 14).

(a) Plant height

In R_2 generation of *Lens* one normal plant on segregation gave two mutants and 25 normal plants. The mutant possessed two primary branches. Out of these one primary branch grew to a height equal to that of control, whereas other branch grew as a dwarf branch. There was no other difference in characters of the two branches.

In *Linum* no such type of branching was observed in R_3 generation.

(b) Height and days to flowering

In R_2 generation of *Lens* a plant was found with two types of branches differing in length. In the branch which was equal to those of the control, days to flowering was decreased, days to flowering was also reduced in the dwarf branches.

TABLE - 13

Spectrum and frequency of viable morphological mutations induced by radiation in R_2 generation of *Lens culinaris* var. JLS-3

Mutant Type	Control	Treatments (gamma rays)					
		5 Kr	10 Kr	15 Kr	20 Kr	25 Kr	30 Kr
1	2	3	4	5	6	7	8
Height							
1. Semi Dwarf	-	-	-	2	1	1	-
2. Dwarf	-	-	3	-	1	1	1
Growth habit							
1. Compact	-	-	-	2	1	2	-
2. Bushy	-	-	2	-	1	1	2
3. Spreading	-	-	-	3	1	1	1
Branching and stem structures							
1. Thin smooth	-	-	1	1	2	2	-
2. Fasciated	-	-	-	2	-	-	1
3. Waxless	-	-	-	1	1	1	1
Leaf structure							
1. Large	-	2	3	1	-	2	1
2. Narrow	-	-	-	2	1	1	1
3. Small	-	-	3	1	-	1	1
4. Boat shaped	-	-	-	1	1	1	2

1	2	3	4	5	6	7	8
Inflorescence							
1. Multi-flor~~a~~etes	-	-	2	1	-	2	1
2. Branches	-	-	-	1	2	1	1
3. Ped~~i~~uncles	-	-	1	2	1	3	1
Flower							
1. Fused	-	3	5	3	1	2	1
2. Fused petal	-	-	-	2	-	2	1
3. Tubular	-	-	-	1	1	1	-
4. Open	-	4	3	1	-	1	2
5. Coupled	-	-	1	2	2	2	1
Fruits							
1. Large	-	1	1	-	1	1	1
2. Small	-	1	-	-	1	-	2
3. Elongated	-	-	1	-	-	-	-
4. Narrow	-	-	-	1	-	1	1
Fertility							
1. Sterile	-	-	2	-	2	-	-
2. Semi-sterile	-	4	-	-	1	1	-
Seed colour							
1. Black	-	-	-	1	2	-	1
2. Brown	-	3	1	-	1	-	1
3. Mottled	-	-	-	1	2	1	-
4. Green-brownish	-	2	-	1	1	-	2
Total	-	20	29	34	28	32	27

TABLE - 14

Spectrum and frequency of viable morphological mutants induced by Radiation in R_2 generation of *Linum usitattissimum* var NP(RR)-5

Mutant type	Control	Treatments (gamma rays)					
		5 Kr	10 Kr	15 Kr	20 Kr	25 Kr	30 Kr
1	2	3	4	5	6	7	8
Height							
1. Semi dwarf	-	-	1	1	-	2	1
2. Dwarf	-	-	2	1	1	1	1
Growth habit							
1. Compact	-	-	-	-	-	1	1
2. Bushy	-	-	1	2	2	1	1
3- Spreading	-	-	-	1	-	1	1
Branching and stem structure							
1. Thin smooth	-	-	-	2	2	1	1
2. Fasciated	-	-	-	-	-	1	-
3. Waxless	-	-	-	-	1	2	1
Leaf structure							
1. Large	-	1	1	1	2	1	1
2. Narrow	-	-	-	1	1	1	1
3. Small	-	1	1	1	1	1	1
4. Boat shaped	-	-	1	-	-	-	-

1	2	3	4	5	6	7	8
<u>Inflorescence</u>							
1. Multi-floretes	-	-	1	1	1	1	1
2. Branches	-	-	-	1	-	1	1
3. Pedincles	-	-	-	-	1	-	1
<u>Flower</u>							
1. Fused	-	1	1	1	1	1	1
2. Fused petal	-	-	-	1	1	1	1
3. Tubular	-	-	-	1	-	1	-
4. Open	-	3	2	1	2	2	1
5. Coupled	-	-	-	1	1	-	1
<u>Fruits</u>							
1. Large	-	1	1	2	1	2	1
2. Small	-	-	1	-	1	1	2
3. Elongated	-	-	-	-	-	1	-
4. Narrow	-	1	-	1	1	1	1
<u>Fertility</u>							
1. Sterile	-	-	1	1	2	2	-
2. Semi-sterile	-	4	-	-	-	1	-
<u>Seed colour</u>							
1. Black	-	-	-	-	1	1	1
2. Brown	-	2	2	2	1	1	1
3. Mottled	-	-	2	1	-	-	-
4. Green brownish	-	-	1	1	1	1	-
TOTAL	-	14	19	25	20	29	24

In R_3 generation of Linum two types of mutants were observed i.e. tall and dwarf in comparison to control but the character of days to flowering was not affected and was equal to control plants.

(c) Colour of seed coat

In R_2 generation, the progeny of one normal R_2 plant produced mutated progeny, the individuals of which possessed bluish testa, whereas other plants developed the normal seed-coat like parental type. On maturity due to bluish testa pods also appeared to be bluish in colour which were easily distinguishable from parental pods. There was no difference in other characters of pods.

In R_3 generation of Linum mutated progeny developed seeds with dark colour of the testa. The plants with darker seed coats resembled the parental plants in all other respect and there was no other notable morphological difference between them except the colour of the seed coat.

Frequency and spectrum of chlorophyll mutations

(i) Frequency of chlorophyll mutations

In both *Lens* and *Linum* frequency of chlorophyll mutations was calculated as percentage of families segregating for any type of chlorophyll mutation (R_2 family basis) and the percentage of chlorophyll mutants isolated in the whole population produced with a particular treatment (R_2 plant basis).

The frequency of chlorophyll mutation in *Lens* and *Linum* is given in tables 15 and 16. Table shows that maximum chlorophyll mutation frequency in *Lens* was 26.08 on the basis of percentage of families and 1.94 on the percentage basis of mutant plants in both *Lens* and *Linum*. As the radiation doses increased the chlorophyll mutation frequency also increased. It was observed that a particular dose is not equally effective on both crops. Dose effect was found to be more pronounced on *Lens* than on *Linum*.

(ii) Spectrum of chlorophyll mutations

The spectrum of chlorophyll mutations was determined by recording each type of chlorophyll mutation and then calculating their relative proportion. The frequencies of each type of chlorophyll mutation in *Lens* and *Linum* are presented in tables 17 and 18.

TABLE - 15

Frequency of chlorophyll mutations in R_2 generation of *Lens culinaris* var. JLS-3 with different doses of gamma rays

Treatment	Dose	R_2 family basis			R_2 mutant basis		
		Total No. of M_2 families raised	No. of R_2 families mutated	% families segregating for mutation	Total No. of plant scored	No. of mutants	% of mutant plants
Control	-	102	-	-	3598	-	-
Gamma rays	5 Kr	72	5	6.94	2398	20	0.834
"	10 Kr	68	8	11.76	1913	29	1.51
"	15 Kr	62	7	11.29	2001	34	1.69
"	20 Kr	60	11	18.33	1972	36	1.82
"	25 Kr	52	11	21.15	2073	38	1.83
"	30 Kr	46	12	26.08	1802	35	1.94

TABLE - 16

Frequency of chlorophyll mutations in R_2 generation of *Linum usitatissimum* var. NP(RR)-5 with different doses of gamma rays

Treatment	Dose	R_2 family basis			R_2 mutant basis		
		Total No. of R_2 families raised	No. of R_2 families mutated	% families segregating for mutation	Total No. of plants scored	No. of mutants	% of mutant plants
Control	-	110	-	-	3235	-	-
Gamma rays	5 Kr	80	3	3.75	2120	11	0.51
"	10 Kr	72	4	5.55	1941	14	0.73
"	15 Kr	67	6	8.95	1842	16	0.86
"	20 Kr	61	9	14.75	1351	21	1.55
"	25 Kr	55	9	16.38	1262	22	1.74
"	30 Kr	42	8	19.09	1236	23	1.86

TABLE - 17

Relative frequency and spectrum of different types of chlorophyll mutations in R_2 generation of *Lens culinaris* var. JLS-3 treated with different doses of gamma rays

Treatment	Dose	Total No. of mutants	Relative occurrence			
			Albina	Chlorina	Xantha-alba	Viridis
Control	-	-	-	-	-	-
Gamma rays	5 Kr	20	-	2	10 (2.0)	8 (40.0)
"	10 Kr	29	1 (3.4)	3 (10.3)	11 (37.9)	14 (48.2)
"	15 Kr	34	1 (2.9)	8 (23.5)	19 (55.8)	6 (17.6)
"	20 Kr	28	2 (7.1)	10 (35.7)	10 (35.7)	6 (21.4)
"	25 Kr	32	1 (3.1)	8 (25.9)	12 (37.5)	11 (34.3)
"	30 Kr	27	1 (3.7)	7 (25.9)	13 (48.1)	6 (22.2)

TABLE - 18

Relative frequency and spectrum of different types of chlorophyll mutations in R_2 generation of *Linum usitattissimum* var. NP(RR)-5 treated with different doses of gamma rays

Treatment	Dose	Total No. of mutants	Relative Occurrence			
			Albina	Chlorina	Xantho-alba	Viridis
Control	-	-	-	-	-	-
Gamma rays	5 Kr	14	-	-	-	-
"	10 Kr	19	-	-	-	-
"	15 Kr	25	-	4 (16.0)	13 (52.3)	6 (32.0)
"	20 Kr	20	-	12 (60.0)	5 (15.0)	5 (25.0)
"	25 Kr	29	-	14 (48.1)	8 (27.5)	7 (24.0)
"	30 Kr	29	2 (8.3)	8 (33.3)	7 (29.1)	7 (29.1)

In both genera four types of chlorophyll mutations were observed i.e. Albina, Chlorina, Xantha, alba and Viridis. The highest relative frequencies of Albina were 7.1 in *Lens* under 20 Kr dose and 8.3 in *Linum* under 30 Kr dose. The chlorina mutation had the maximum relative proportion in *Lens* (60.0) and in *Linum* (35.7) under the same dose 20 Kr. It was followed by Xantha -alba both in *Lens* (55.8) and in *Linum* (52.3) under 15 Kr dose, Viridis 34.3 in *Lens* and 24.0 in *Linum* under 25 Kr doses and Albina 3.7 in *Lens* and 8.3 in *Linum* under 30 Kr dose.

Effectiveness and efficiency of gamma irradiation

Effectiveness of irradiation usually means the rate of mutations as related to dose, while efficiency is generally referred to mutation rate in relation to other biological effects induced and is considered as measure of damage.

The values of effectiveness and efficiency of different doses of gamma rays, to which the two genera were subjected are recorded in tables 19 & 20.

<u>Effectiveness of gamma rays</u>

Table 19 and 20 indicate that for both genera doses higher than 30 Kr proved to be most effective. Highest effectiveness was 2.31 in *Lens* and 1.95 in *Linum* under the same 30 Kr dose. In both genera effectiveness decreased with decrease of doses of gamma rays.

<u>Efficiency of gamma rays</u>

It is evident from the table 19 and 20 in both genera that efficiency of gamma rays increased with an increase in dose rate except 10 Kr and 15 Kr doses in *Linum* where 10 Kr dose showed higher and 15 Kr lower efficiency.

Thus in general, increase in biological damage was accompanied with a parallel increase in mutation rate. A comparison of efficiency and ~~of~~ effectiveness of various doses of gamma rays indicate that *Lens* happens to be more sensitive to gamma rays than *Linum*.

TABLE - 19

Efficiency and effectiveness of different doses of gamma rays on *Lens culinaris* var. JLS-3

Treatment	Dose	Percentage of lethality (L)	Percentage of M_1 plant progenies segregating (Mpl)	Efficiency of gamma rays	Effectiveness of gamma rays
Control	-	0.00	-	-	-
Gamma rays	5 Kr	28.5	8.15	0.28	1.63
"	10 Kr	42.0	18.25	0.43	1.82
"	15 Kr	60.0	31.05	0.51	2.07
"	20 Kr	62.0	43.65	0.70	2.18
"	25 Kr	65.0	56.62	0.87	2.26
"	30 Kr	70.0	69.58	0.99	2.31

TABLE - 20

Efficiency and effectiveness of different doses of gamma rays on *Linum usitatissimum* var. NP(RR)-5

Treatment	Dose	Percentage of lethality (L)	Percentage of M_1 plant progenies segregating (Mpl)	Efficiency of gamma ray	Effectiveness of gamma rays
Control	-	-	-	-	-
Gamma rays	5 Kr	2.0	4.95	2.48	0.99
"	10 Kr	8.0	10.98	1.37	1.09
"	15 Kr	20.0	19.25	0.96	1.28
"	20 Kr	28.0	28.63	1.02	1.43
"	25 Kr	33.0	39.12	1.18	1.56
"	30 Kr	42.0	58.60	1.39	1.95

Induced mutagenesis, quantitative estimates

In order to ascertain the variability of traits leading to mutational improvements, quantitative estimates were undertaken. Seeds of *Linum* and *Lens*, the former being oily and the latter proteinaceous, were subjected to different doses of gamma-rays, for determining the quantum and behaviour of induced micromutational changes with respect to the following parameters.

1. Days to flowering.
2. Average number of branches per plant.
3. Average number of fruits per plant.
4. Weight of 1000 seeds.
5. Seed output per plant.

The magnitude of induced variations was estimated through variance while behaviour was determined with the help of mean heritability and response to selection. The data collected is described hereunder and the same is summarised in tables 21 to 30.

1. Days to flowering

(a) Behaviour of mean: Tables 21 and 22 reveal the mean about 'days to flowering'. It can be visualised that with almost all the doses of gamma-rays the mean of their character declined with the exception of 5 Kr dose with which it was equal to the control (Fig. 16).

With higher doses of gamma rays the decrease in mean was more significant while with lower doses the difference was not significant as compared to the controls.

(b) __Variance__: It is evident from tables 22 and 23 that 'days to flowering' ranged from 87 to 116 in __Lens__ with 30 Kr dose against 94 - 110 of the control while in __Linum__ it ranged from 87 to 118 with 15 Kr dose, range of its control was 100-115. With all the doses 7 days early and 9 days late flowering was induced in __Lens__ while in __Linum__ 2 days early and 9 days late flowering was induced.

Overall variance in __Lens__ was the highest (28.3) with 30 Kr gamma rays as compared to the control (10.5). With other doses of gamma rays the overall variance was also comparatively higher except with 5 Kr dose where it was found to be comparatively lower than its control. Fig. 16 clearly indicates

TABLE - 21

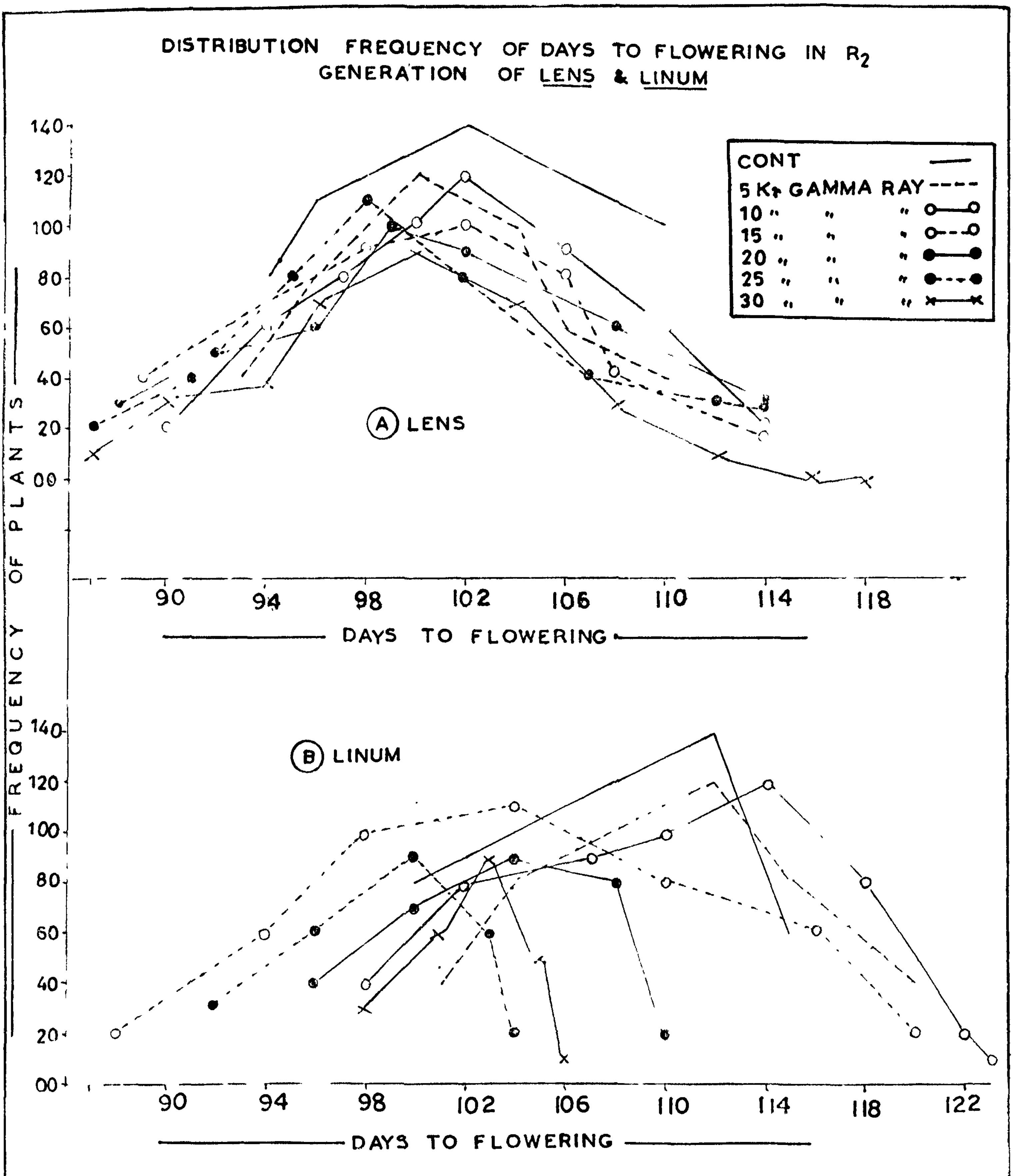
Range, mean, overall variance, components of variation and genetic parameters for days to flowering in R_2 generation of *Lens culinaris* var. JLS-3

Treatment	Dose	Range	Mean	Overall variance	PCV %	GCV %	Herita-bility %	Genetic advance as % of mean
Control	-	94-110	103.39	10.50	4.78	2.68	31.18	3.06
Gamma rays	5 Kr	93-110	102.18	8.43	3.81	2.11	30.10	2.41
"	10 Kr	90-114	101.72	22.92	8.21	6.40	60.50	10.29
"	15 Kr	89-114	100.88	20.18	6.97	4.82	48.11	6.81
"	20 Kr	88-114	101.82	26.72	9.41	6.80	51.68	10.10
"	25 Kr	87-114	101.70	25.17	8.18	6.12	56.89	9.26
"	30 Kr	87-116	101.40	28.23	9.31	7.93	65.15	12.23

TABLE - 22

Range, mean, overall variance, components of variation and genetic parameters for days to flowering in R_2 generation of *Linum usitattissimum* NP(RR)-5

Treatment	Dose	Range	Mean	Overall variance	PCV %	GCV %	Heritability %	Genetic advance as % of mean
Control	-	100-115	109	12.3	4.23	2.21	30.92	2.96
Gamma rays	5 Kr	101-118	109	14.3	4.81	2.32	29.82	2.24
"	10 Kr	98-121	107	14.9	6.20	4.34	30.91	3.29
"	15 Kr	87-118	105	15.6	6.92	4.83	38.24	4.18
"	20 Kr	98-110	102	17.9	8.31	5.92	40.12	5.32
"	25 Kr	93-105	101	18.3	8.92	5.81	42.32	5.61
"	30 Kr	98.106	101	20.3	8.23	6.30	49.91	8.28



DISTRIBUTION FREQUENCY OF DAYS TO FLOWERING IN R_2 GENERATION OF LENS & LINUM
CONT
5 Kr GAMMA RAY
10 " " "
15 " " "
20 " " "
25 " " "
30 " " "
FREQUENCY OF PLANTS
140
120
100
80
60
40
20
00
(A) LENS
90
94
98
102
106
110
114
118
DAYS TO FLOWERING
(B) LINUM
90
94
98
102
106
110
114
118
122
DAYS TO FLOWERING

the fact that variability followed both upward and downward trends with respect to 'days to flowering'.

In *Linum* also highest overall variance was observed with 30 Kr dose of gamma rays. Other doses too induced higher variance as compared to its control. Fig. 16 shows that variability to its control and it occurred with respect to both early and late flowering.

With the exception of 5 Kr, all doses of gamma rays induced higher phenotypic variability in *Lens* (Table 21). Induced genotypic variability was also found higher with all the doses of gamma rays except 5 Kr and 10 Kr doses.

In *Linum* phenotypic and genotypic variabilities were higher with all the doses of gamma rays. Highest PCV was recorded with 25 Kr dose while highest GCV was recorded with 30 Kr dose as compared to the control (Table 22).

(c) Heritability and genetic advancement

Heritability induced by gamma rays has been found to be significantly higher in both *Lens* and *Linum* except with 5 Kr dose in *Lens* and 5 and 10 Kr doses in *Linum* (Tables 21 and 22).

Genetic advancement in both *Lens* and *Linum* was also significantly higher with higher doses of gamma rays. Highest genetic advancement was induced by 30 Kr dose in both the plants as Tables 21 and 22 clearly indicate~~s~~.

2. Average number of branches per plant

Table 13 indicates that with all the doses of gamma rays the mean for average number of branches in *Lens* decreased as compared to its control. On *Linum* only 20, 25 and 30 Kr doses of gamma rays were tried. With 25 Kr dose the minimum (mean) number of branches were produced while the mean maximum number of branches (4.9) were produced with 30 Kr dose. With 20 Kr dose the mean was lower than that of 30 Kr but higher, compared to 25 Kr. It is evident from the table that the higher doses of gamma rays reduce the mean of number of branches in both the taxa (Tables 23 and 24).

(b) Variation (variance)

In case of *Lens* the number of branches ranged from 2 to 10 with 20 and 30 Kr dose against 3-11 in its control. The overall variance was highest (2.51) with 5 Kr dose followed by 10 Kr (2.50), 15 Kr (2.42), 25 Kr (2.32), 20 Kr (2.23) and 30 Kr (2.20). In case of *Linum* the highest overall variance of 2.21 was found with 30 Kr dose, which was followed by 25 Kr (2.12) and 20 Kr (1.39) (Tables 23 and 24).

With all the doses of gamma rays administered, a higher phenotypic variability was recorded in case of *Lens* as compared to its control. Similarly all the doses of gamma rays reduced a higher genotypic variability in *Lens*. Highest PCV (32.11) was obtained with 5 Kr dose while highest GCV (20.30) was obtained with 15 Kr (Fig. 17).

In case of *Linum* highest PCV and GCV were induced by 30 Kr dose which were 21.2 and 10.24 respectively.

(c) Heritability and genetic advancement

Table 13 indicates that except 5 Kr dose all the doses of gamma rays administered, induced significantly higher heritability in *Lens* as compared to its control. Highest heritability (43.72) was recorded with 10 Kr dose followed by 15 Kr (41.15).

TABLE - 23

Range, mean, overall variance, components of variationx and genetic parameters for number of branches per plant in R_2 generation of *Lens culinaris* var. JLS-3

Treatment	Dose	Range	Mean	Overall variance	PCV %	GCV %	Herita-bility %	Genetic advance % of mean
Control	-	3-11	6.54	1.53	19.42	10.83	30.89	12.91
Gamma rays	5 Kr	2-11	6.30	2.51	32.11	17.52	29.69	19.27
"	10 Kr	2-11	6.12	2.50	29.57	19.52	43.72	26.59
"	15 Kr	2-11	6.09	2.42	31.62	20.30	41.15	26.89
"	20 Kr	2-10	5.19	2.23	30.80	17.62	32.62	20.65
"	25 Kr	2-11	5.07	2.32	31.19	17.30	30.23	19.64
"	30 Kr	2-10	5.04	2.20	29.21	17.35	39.92	22.32

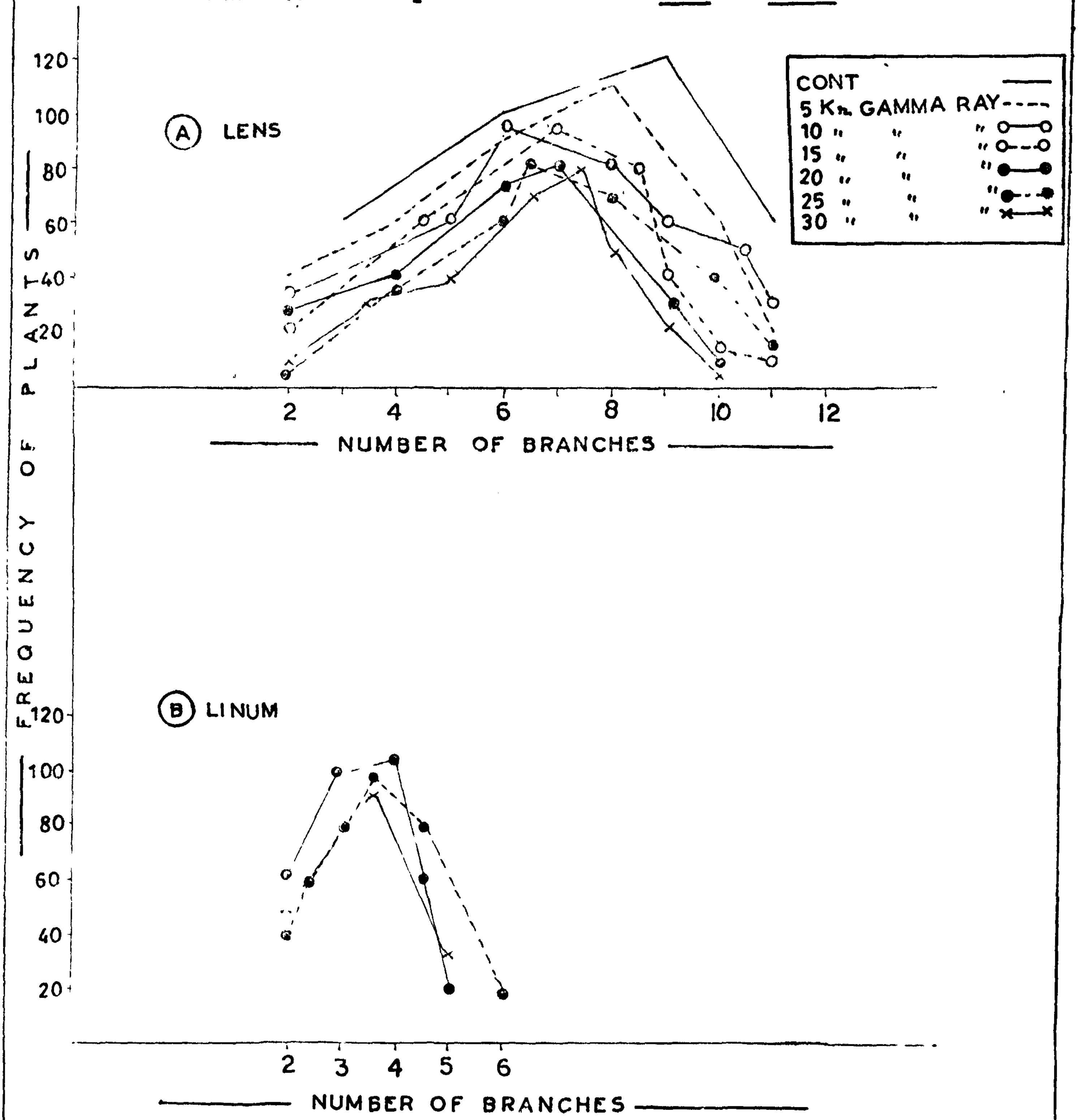
CD 5% 0.24 0.92

TABLE - 24

Range, mean, overall variance, components of variation and genetic parameters for number of branches per plant in R_2 generation of *Linum usitatissimum* var. NP(RR)-5

Treatment	Dose	Range	Mean	Overall variance	PCV%	GCV %	Heritability %	Genetic advance % of mean
Control	-	-	-	-	-	-	-	-
Gamma rays	5 Kr	-	-	-	-	-	-	-
"	10 Kr	-	-	-	-	-	-	-
"	15 Kr	-	-	-	-	-	-	-
"	20 Kr	2-5	4.8	1.39	17.34	9.39	26.28	10.12
"	25 Kr	2-6	3.9	2.12	19.46	9.82	30.26	11.32
"	30 Kr	2-5	4.9	2.21	21.12	10.24	32.42	12.12
	CE 5%	0.04		0.62				

DISTRIBUTION FREQUENCY OF NUMBER OF BRANCHES PERPLANT IN R_2 GENERATION OF LENS & LINUM



In case of <u>Linum</u>, highest heritability of 32.42 was induced by 30 Kr gamma rays followed by 25 Kr (30.26).

The percentage of mean of genetic advancement indicates in case of <u>Lens</u> that it was significantly higher with all the doses of gamma rays used. It was highest with 15 Kr (26.89) followed by 10 Kr (26.59). In case of <u>Linum</u> the highest genetic advancement was induced by 30 Kr dose (12.12) (Fig. 17).

3. <u>Average number of fruits per plant</u>

(a) <u>Behaviour of mean</u>

In the controls of <u>Lens</u> and <u>Linum</u> the range of fruits number per plant was 61 - 191 and 4 - 5 respectively with 103.18 and 4.82 as their mean values (Table 25-26). In case of <u>Lens</u> the value of mean was slightly increased with 5 Kr and 10 Kr doses in comparison with the control but with all other doses administered, it decreased. The decline was much more significant with 20, 25 and 30 Kr doses. In <u>Linum</u> also the 5 Kr dose induced an increase in the mean value for number of fruits per plant but with the remaining five doses the value of mean was decreased.

In this taxon also 20, 25 and 30 Kr doses decreased the mean significantly.

(b) <u>Variance</u>

In case of <u>Lens</u>, the overall variance was higher with all the doses of gamma rays as compared to the control. The highest overall variance (525.38) was recorded with 15 Kr dose. In case of <u>Linum</u> 5 Kr dose lowered ~~to~~ overall variance in comparison to the control, but all other doses increased. Overall variance (69.13) in <u>Linum</u> was induced by 30 Kr. The higher doses <u>i.e.</u> 20, 25 and 30 Kr doses induced comparatively higher overall variance than the three lower doses as Table 14 reveals.

All the doses of gamma rays induced higher phenotypic variability (PCV) in <u>Lens</u> as compared to its control. Highest PCV (32.81) was induced by 30 Kr doses. In case of <u>Linum</u> phenotypic variability with 5 Kr dose was found lower than its control, while the other five doses induced comparatively higher phenotypic variability. Highest phenotypic variability (6.96) in <u>Linum</u> was recorded with 30 Kr dose.

In both *Lens* and *Linum* all the doses of gamma rays administered induced higher genotypic variability. The highest GCV was obtained with 5 Kr in the former (20.32) and with 30 Kr in the case of the latter (15.12) (Fig. 18).

Heritability and genetic advancement

In case of *Lens*, 5 Kr, 15 Kr and 20 Kr doses induced higher heritability while with 10, 25 and 30 Kr doses the heritability was found lowered. Highest heritability of 48.60% was induced by 5 Kr dose.

In case of *Linum* all the doses of gamma rays administered, induced higher heritability as compared to the control. Higher heritability in *Linum* (26-29) was induced by 30 Kr dose (Table 25-26).

Genetic advancement was found enhanced with all the doses of gamma rays in both *Lens* and *Linum*. Highest genetic advancement (29.40) was induced by 5 Kr dose in the former and by 30 Kr dose in the latter (24.13).

TABLE - 25

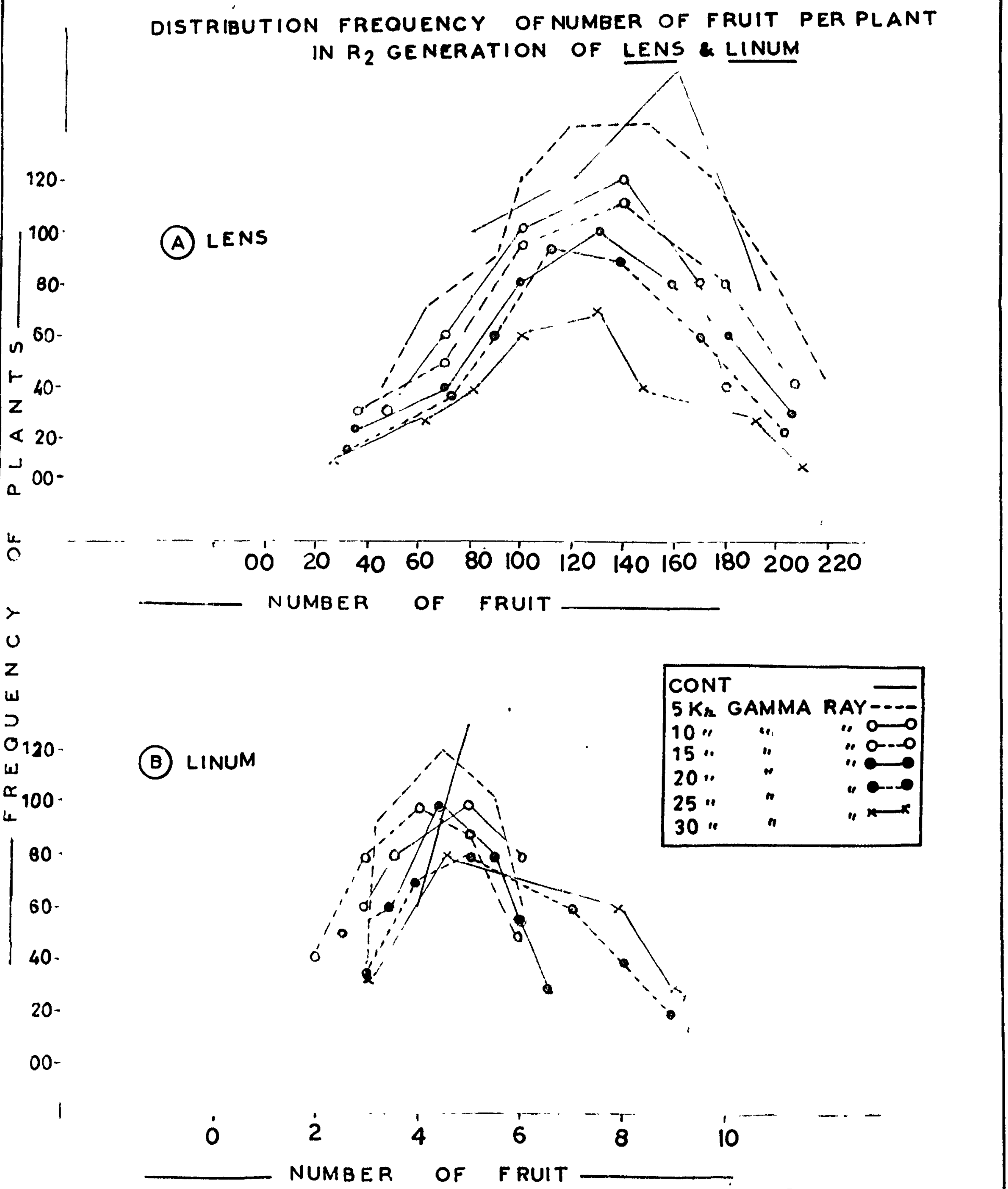
Range, mean, overall variance, components of variation and genetic parameters for numbers of fruits per plant in R_2 generation of *Lens culinaris* var. JLS-3

Treatment	Dose	Range	Mean	Overall variance	PCV %	GCV %	Herita-bility %	Genetic advance as % of mean
Control	-	61-191	103.18	318.72	17.25	9.49	30.29	10.35
Gamma rays	5 Kr	46-220	105.38	491.13	29.32	20.32	48.60	29.40
"	10 Kr	48-180	107.39	419.34	20.19	10.19	25.12	10.92
"	15 Kr	36-208	103.11	525.38	28.52	18.21	40.69	23.82
"	20 Kr	35-207	96.72	501.49	31.10	17.92	33.92	21.32
"	25 Kr	33.204	96.89	429.47	26.91	11.26	28.99	21.23
"	30 Kr	29.210	96.32	499.79	32.81	17.38	28.24	21.92
		CD 5%	4.40	10.08				

TABLE - 26

Range, mean, overall variance, components of variation and genetic parameters for number of fruit per plant in R_2 generation of *Linum usitattissimum* var. NP (RR)5

Treatment	Dose	Range	Mean	Overall variance	PCV %	GCV %	Herita-bility %	Genetic advance as % of mean
Control	-	4-5	4.82	40.28	4.16	3.20	12.32	3.12
Gamma rays	5 Kr	3-6	4.92	39.62	4.13	3.41	14.24	4.32
"	10 Kr	3-6	4.12	52.36	5.12	9.36	19.62	9.23
"	15 Kr	2-6	4.10	51.96	5.19	12.12	21.42	12.18
"	20 Kr	3-7	3.96	60.16	5.92	13.32	24.36	14.19
"	25 Kr	3-9	3.91	63.24	6.12	14.92	24.92	21.09
"	30 Kr	3-9	3.80	69.13	6.96	15.12	26.29	24.13
		CD 5%	1.40	7.08				



DISTRIBUTION FREQUENCY OF NUMBER OF FRUIT PER PLANT IN R2 GENERATION OF LENS & LINUM
(A) LENS
FREQUENCY OF PLANTS
120
100
80
60
40
20
00
00 20 40 60 80 100 120 140 160 180 200 220
NUMBER OF FRUIT
(B) LINUM
120
100
80
60
40
20
00
0 2 4 6 8 10
NUMBER OF FRUIT
CONT
5 Kr. GAMMA RAY
10 " " "
15 " " "
20 " " "
25 " " "
30 " " "

Weight of 1000 seeds

(a) Behaviour of the mean

Weight of 1000 seeds ranged between 20.08 and 20.12 in the controls of *Lens* while in the controls of *Linum* the range was 14.82 to 20.16. Their respective means were 20.80 and 14.29 (Tables 27 and 28). In case of *Lens*, the value of mean was slightly increased (20.82) by 10 Kr dose but with the remaining five doses the value of mean was found decreased. In case of *Linum*, all the six doses administered, increased the value of mean. The highest value of the mean (19.92) was obtained with 15 Kr dose.

(b) Variance

The overall variance in the controls of *Lens* and *Linum* were 0.79 and 0.62 respectively. Variance was found increased with all the doses of gamma rays administered in both *Lens* and *Linum*. Highest overall variance of 1.41 was induced by 20 Kr in the former and 1.39 by 30 Kr dose in the latter (Table 27 and 28).

In case of *Lens*, phenotypic variability (PCV) was higher with all the doses of gamma rays as compared to the control but in *Linum* 5 Kr dose slightly decreased the PCV, while all the other doses increased it. Highest PCV in *Lens* (7.83) was induced by 20 Kr dose of gamma rays. In *Linum* the highest phenotypic variability of 7.10 was induced by 30 Kr dose.

Genetic variability (GCV) in the controls of *Lens* and *Linum* was 1.46 and 0.96 respectively. In case of *Lens* 5 Kr dose decreased the GCV but all other doses increased it. Highest GCV in Lens (4.23) was recorded with 20 Kr dose. In case of *Linum*, genetic variability was found increased with all the doses administered. The higher GCV of 2.12 in *Linum* was induced both by 25 Kr and 30 Kr doses.

(c) Heritability and genetic advancement

The heritability in the controls of *Lens* and *Linum* was 4.82 and 2.97 (Tables ~~15~~ 27&28.) but the administration of 5 Kr dose decreased it to 4.47 and 2.92 respectively. The remaining five doses increased the heritability in both the plants. The higher heritability of 23.0% was induced by 25 Kr dose in *Lens*. In *Linum* the highest heritability of 5.12% was induced by 30 Kr dose.

TABLE - 27

Range, mean, overall variance, components of variation and genetic parameters for weight of 1000 seeds in R_2 generation of *Lens culinaris* var JLS-3

Treatment	Dose	Range	Mean	Overall variance	PCV %	GCV %	Herita-bility %	Genetic advance as % of mean
Control	-	20.08-20.12	20.80	0.79	5.41	1.46	4.82	0.81
Gamma rays	5 Kr	20.15 22.30	20.72	1.12	5.42	1.12	4.47	2.50
"	10 Kr	20.10 22.27	20.82	1.21	6.12	2.23	12.29	1.72
"	15 Kr	20.50 22.31	20.78	1.19	5.72	2.92	13.221	2.24
"	20 Kr	19.80 22.45	20.79	1.41	7.83	4.23	14.89	2.19
"	25 Kr	19.79 22.54	20.12	1.21	6.92	4.21	23.0	3.12
"	30 Kr	19.82 22.51	20.11	1.28	6.18	3.47	22.98	3.81
		CD	5%	0.19	0.74			

TABLE - 28

Range, mean, overall variance, components of variations and genetic parameters for weight of 1000 seeds in R_2 generation of *Linum usitatissimum* var. NP(RR)-5

Treatment	Dose	Range	Mean	Overall variance	PCV %	GCV %	Herita-bility	Genetic advance as % of mean
Control	-	14.82 20.16	14.29	0.62	4.82	0.96	2.97	0.49
Gamma rays	5 Kr	15.78 22.32	15.12	0.98	4.79	1.12	2.92	0.52
"	10 Kr	18.32 22.42	19.39	1.11	6.32	1.32	3.12	1.26
"	15 Kr	17.92 24.32	19.92	1.12	6.19	1.42	3.98	1.98
"	20 Kr	17.96 24.24	19.89	1.32	6.12	1.89	4.12	2.01
"	25 Kr	17.92 24.32	19.82	1.36	6.19	2.12	4.81	2.92
"	30 Kr	16.92 24.82	19.80	1.39	7.10	2.12	5.12	2.97
		CD 5%	0.11	0.54				

A comparison with their controls reveals that all the doses of gamma rays administered, induced higher genetic advancement in both *Lens* and *Linum*. The highest mean values of genetic advancement 3.81 and 2.97 were induced by 30 Kr gamma rays in *Lens* and *Linum* respectively.

5. Seed yield

(a) Behaviour of the mean

Table 29 reveals that the mean seed yield of *Lens* decreased with all the doses of gamma rays administered against 3.50 of its control. It was found to be inversely correlated with the dose rate. In case of *Linum* table 30 the mean seed yield of the control was 8.7 but it was increased to 8.8 with the administration of 5 Kr dose while with the rest of the five doses it was progressively decreased following an inverse correlation with the dose rate.

(b) Variance

The overall variance in the control of *Lens* was 0.43 but it was found to be reduced to 0.38 with 5 Kr gamma rays. With the higher doses overall variance was increased. Highest overall variance of 0.53 in *Lens* was obtained both with 15 Kr and 30 Kr doses. In case of *Linum* the control recorded 0.82 overall variance and all the doses of gamma rays administered decreased the overall variance as is evident from table 30.

In case of *Lens* lower phenotypic variability (PCV) was induced by 5 Kr and 20 Kr doses while with 10, 15, 25 and 30 Kr doses the PCV was found to be significantly increased. Highest PCV of 29.98 was obtained with 30 Kr dose. In *Linum* the phenotypic variability progressively increased with the increasing dose rate. Highest phenotypic variability of 27.32 in *Linum* was recorded with 30 Kr dose.

Genetic variability (GCV) in *Lens* reduced to 0.32 by 5 Kr dose as compared to 8.90 of its control. With the remaining five doses the GCV was significantly increased. Highest GCV of 18.50 in *Lens* was recorded with 20 Kr dose. In *Linum* all the doses of gamma rays administered, increased the genotypic variability to a greater or lesser extent. Highest GCV of 14.98% in *Linum* was recorded with 30 Kr dose (Fig. 19).

TABLE - 29

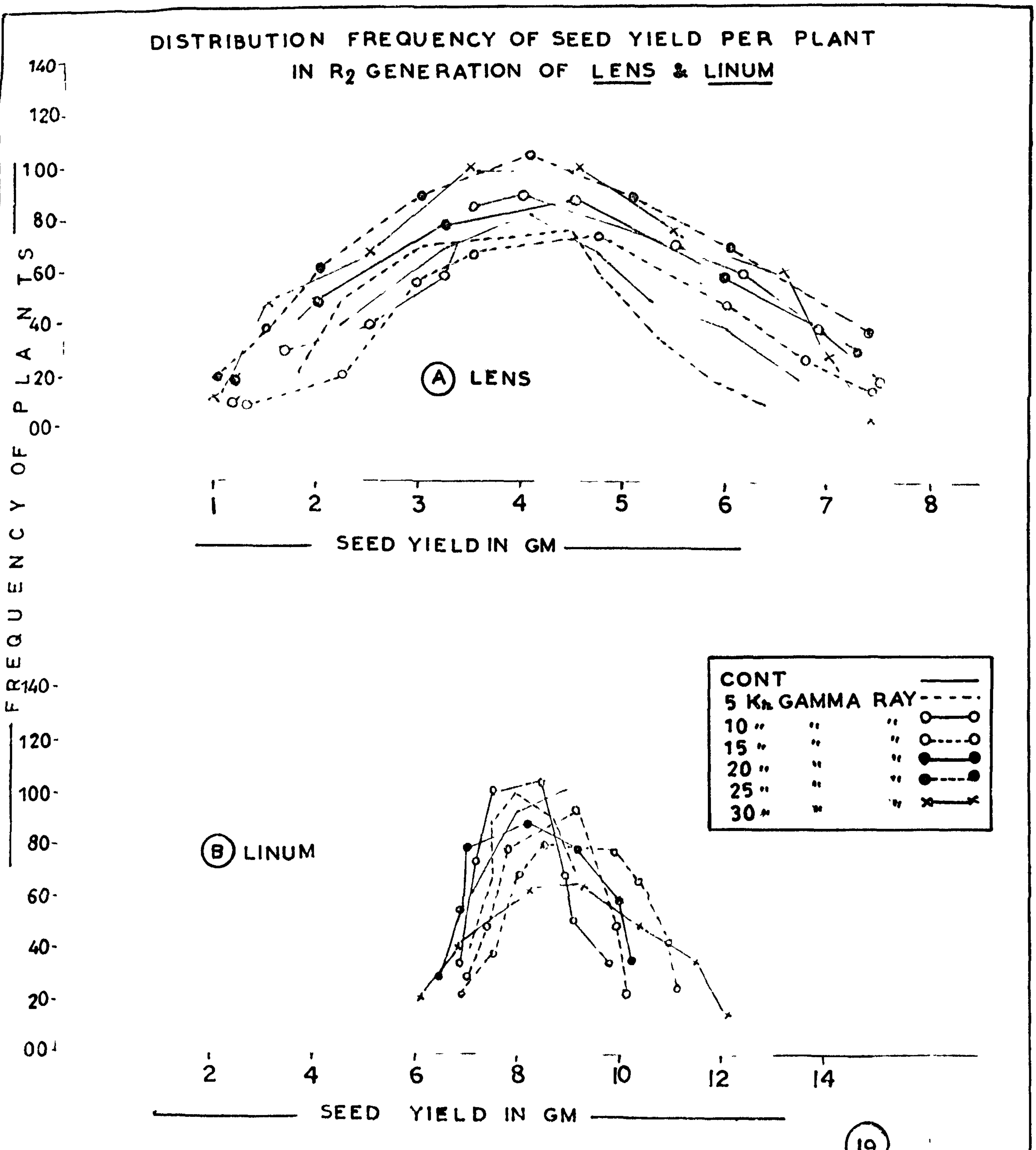
Range, mean, overall variance, components of variation and genetic parameters for seed yield in R_2 generation of *Lens culinaris* var. JLS-3

Treatment	Dose	Range	Mean	Overall variance	PCV %	GCV %	Heritability %	Genetic advance as % of mean
Control	-	2.28 6.72	3.50	0.43	20.18	8.90	19.42	8.08
Gamma rays	5 Kr	1.80 6.42	3.32	0.38	15.03	0.32	34.82	13.22
"	10 Kr	1.26 7.50	3.24	0.51	29.42	17.32	27.32	17.20
"	15 Kr	1.32 7.42	3.21	0.53	20.89	10.49	24.62	19.30
"	20 Kr	1.25 7.34	3.18	0.48	17.92	18.50	26.52	18.12
"	25 Kr	1.10 7.40	3.14	0.52	23.53	17.89	29.29	20.19
"	30 Kr	1.15 7.48	3.13	0.53	20.98	16.92	32.12	14.96
		CD 5%	0.17	0.63				

TABLE - 30

Range, mean, overall variance, components of variation and genetic parameters for seed yield per plant in R_2 generation of *Linum usitatissimum* var. NP (RR)-5

Treatment	Dose	Range	Mean	Overall variance	PCV %	GCV %	Herita-bility	Genetic advance as % of mean
Control	-	7.2-8.9	8.7	0.82	17.3	6.92	13.24	7.32
Gamma rays	5 Kr	7.1-9.2	8.8	0.79	17.98	7.10	14.32	7.52
"	10 Kr	7.0-9.8	8.2	0.59	19.78	9.32	15.72	11.12
"	15 Kr	6.8-10.2	8.0	0-60	20.42	9.89	18.20	11.97
"	20 Kr	6.5-10.5	7.92	0.68	22.32	12.12	18.98	12.12
"	25 Kr	6.8-11.2	7.82	0.63	24.30	14.23	19.29	13.32
"	30 Kr	6.1-12.2	7.32	0.72	27.32	14.98	20.12	13.80
		CD 5%	0.12	0.42				



DISTRIBUTION FREQUENCY OF SEED YIELD PER PLANT
IN R_2 GENERATION OF LENS & LINUM
FREQUENCY OF PLANTS
140
120
100
80
60
40
20
00
(A) LENS
1
2
3
4
5
6
7
8
SEED YIELD IN GM
CONT
5 Kr. GAMMA RAY
10 " " "
15 " " "
20 " " "
25 " " "
30 " " "
(B) LINUM
140
120
100
80
60
40
20
00
2
4
6
8
10
12
14
SEED YIELD IN GM

(19)

(c) Heritability of genetic advancement

Higher heritability was induced in both *Lens* and *Linum* by all the doses of gamma rays administered as compared to their controls. In *Lens* higher heritability of 34.82% was obtained with 5 Kr dose while in *Linum* it was 20.12% obtained with 30 Kr dose.

All the doses of gamma rays administered also induced comparatively higher genetic advancement in both the plants. In *Lens* the highest mean genetic advancement of 20.19% was induced by 25 Kr dose while in *Linum* it was 13.80% induced by 30 Kr gamma rays (Tables 29 and 30, Fig. 19).

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DISCUSSION

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D I S C U S S I O N

Enhancement of mutation frequency and alteration of mutational events in the desired direction are the two major goals of mutation research. Mutation breeding has been used in the recent years as a valuable supplement to other methods of plant breeding for developing better varieties with new plant architecture, superior biochemical constitution and suitable growth and development rhythms. The utility of this method is evident from the fact that in several crops, induced mutations have directly released new varieties such as Reimea rice mutant with reduced straw length and higher yield stability developed in Japan, Sharbati Sonora - an amber grain mutant of wheat developed in India, Meri - an early maturing barley mutant developed in Sweden and Luther - a non-lodging barley mutant reported in the Washington State U.S.A. (Sigurbjernson and Minke, 1969) are some of the examples showing achievements of mutation breeding.

Radiation induced variations, in breeding is a field, where the practical gains would grow with the additions in our understanding about the basic informations on the radiation sensitivity, standardisation of doses, frequency and spectrum of mutations, mutagenic effectiveness and efficiency etc. This applies specially to the crops like _Lens_ and _Linum_ where the systematic radiation studies to induce variability are lacking.

The present investigations on two common cultivars of _Lens culinaris_ (variety JLS-3) and _Linum usitattisimum_ (variety NP (RR)-5) were carried on with two main objectives. Firstly, it was intended to compare the effect of gamma rays, on an oily and proteinaceous crop plant. Being the first systematic attempt, this study included the standardisation of doses and estimation of relative radiation sensitivity of the two crop plants. Secondly, it was planned to study the quantum and the direction of micromutational variability for some characters of economic importance.

i) Germination

It is evident from the present study that the radiation treatments given to the varieties of *Lens* and *Linum* inhibited, the germination process compared to control in linear proportion to the intensity of radiations. Inhibitory effect of radiation has been reported by several workers in different group of plants (Gregory, 1955 and 1968; Sjodin, 1962; Santos, 1965; Raghuvanshi and Singh, 1977; Chaghtai *et al*., 1978 a, b). On the basis of the results obtained in the present study it may be inferred that *Lens* is more sensitive to gamma ray treatments than *Linum*.

Abidi *et al*., (1979) found that gamma rays when applied to dry seeds, promoted the germination process in *Linum*. Present work, however, has revealed that gamma rays do not exercise a similar promoting effect in pre-soaked condition.

It appears that pre-soaking renders the seeds vulnerable to the effect of irradiation and that the germination is inhibited due to biochemical interference of radiation with the physiological activities involved in the process of seed germination in both the crops in different degree.

ii) Growth rate

In addition to seed germination, the two growth parameters i.e. rate of shoot and root elongation have also been found to correlate with the intensity of radiation dosage. It affected the growth rate of *Linum* mildly in comparison to *Lens* (Tables 2 and 3).

Growth inhibition in crop plants under the influence of various radiation doses has been a common observation and it was reported by Gunckel and Sparrow (1961), Santos (1965), Davies (1968), Rai (1971), Nayer (1971), Chopra (1972) and Raghuvanshi and Singh (1977), Sinha and Bose (1978). The results of the present work are, therefore, in agreement with the earlier findings.

The growth rate showed a gradual recovery from the inhibitory effect of radiation as it increased with the advance of time period after giving treatments. With respect to growth rate in *Lens* it has been found that it is more affected than *Linum*, although in both dwarf mutants were produced due to stunted growth.

Different workers hold different opinions regarding the phenomenon on stunted growth to mention a few, the following are quoted:

i) Uneven damage of meristimatic cells due to genetic injuries (Gray and Scholes, 1951; Lea, 1955).

ii) Marked decrease in auxin level (Gordan, 1954 and 1957).

iii) Marked effect on auxin synthesis (Gunckel and Sparrow, 1961).

iv) Effect on respiratory enzymes (Bjornseth et al., 1957).

v) Effect on mitosis, as well as physiological disorders caused by radiation both of which may be responsible for stunted growth.

iii) Survival

Seedling survival decreased in both the cases with increasing doses of gamma ray (Tables 2 and 3) indicating a clear correlation between the dose and the survival of plants.

In the present study on survival *Lens* has been found to be more sensitive to radiation than *Linum*.

The fall in survival following irradiation treatment has been reported by a number of workers and the results obtained in the present investigation in this regard are, therefore, not uncommon (Varghese and Swaminathan, 1968; Bari, 1971; Hussein and Disouki, 1976).

iv) Chlorophyll deficiency and morphological abnormalities

Appearance of chlorophyll chimeras and other morphological abnormalities in R_1 generation of both plants investigated has been found to show a direct correlation with dose rate of the gamma rays. Higher the dose of radiation, greater was the frequency of such abnormalities.

Frequency of gamma ray induced variation was higher in *Lens* in comparison to *Linum*. Most of the chimeras did not survive. Though the genetic causes for such a phenomenon could not be explored and ascertained, it may be conjectured that a mutation at the gene level may have caused restricted synthesis of chlorophyll which obviously hampered the metabolic activity leading to the semi-starvation condition resulting in the death of chimeras. It is notable that R_1 chlorophyll chimeras obtained with gamma ray treatments produced a markedly higher number of mutants in R_2 in Lens than in Linum. The occurrence of chlorophyll deficiency in different crop plants following the treatments with radiation agents has been reported by many workers (Hussein et al., 1974; Gupta, 1976; Chaghtai and Hassan, 1978 a). Results of the present study confirm the findings of the previous workers.

a) Leaf abnormalities

Leaf abnormalities were induced in R_1 progenies of both the plants with different doses of gamma rays, and these were transmitted to R_2 generation, showing that leaf abnormalities induced by these treatments affect the genetic material and hence transmitted in the subsequent generation. However leaf abnormalities induced by comparatively higher doses of gamma rays were transmitted in R_2 generation which has also been reported in different crops (Chauhan, 1969; Gupta, 1976; Sharma and Sharma 1979). Frequency of plants possessing abnormal leaves was higher in Lens than in Linum.

b) Branching

In both the cases axillary branching was induced by gamma rays: This may be due to the damage caused to the apical meristems by the treatment, under such circumstances the lateral branching is invariably get induced.

(c) Flower

Floral abnormalities, like change in symmetry from zygomorphy to actinomorphy in Lens and from Actionomorphy to Zygomorphy in Linum is induced. In Lens more such abnormalities were recorded than in Linum in the present study.

vi) Pollen sterility

In both the crops pollen sterility was induced with the increased dose level of irradiation (tables 2 and 3). Pollen sterility was more pronounced in *Lens* than in *Linum*. In both genera highest pollen sterility was observed with 30 Kr dose of gamma rays.

It may be postulated that radiation brings about qualitative changes either in the cytoplasm or in genes to alter their normal behaviour which on interaction induce pollen sterility. Pollen sterility has been reported to be a rather common by - product of radiation treatment (Shivaraj *et al.*, 1962; Shivraj and Ramana Rao, 1965; Ragers and Xavier, 1972; Hussein and Disouki, 1976).

vii) Seed output

Seed output in both cases has been observed to be markedly affected under the influence of higher doses of gamma rays. It shows an inverse linear correlation with dose rate. A comparison of seed output of treated plants from the controls of the two crops studied indicates that in plants of *Lens* seed yield was very poor, as the radiation had more effect on seed output of *Lens* than of *Linum*.

Size of the seeds in treated progenies of both the crop plants was markedly diminished and their weight was also decreased, with the increase of radiation doses. Here again *Lens* was found to be highly affected in comparison to *Linum*. Bari (1971) and Badwal *et al.*,(1972) have reported abnormalities regarding seed-morphology of flax induced by irradiation.

Gamma ray sensitivity of the two crops

So far as the gamma ray sensitivity of the two crops is concerned, it has been found that the proteinaceous *Lens* is more sensitive to radiation than the oily *Linum*.

The degree of sensitivity of different doses of gamma ray also varies in the two crops (Table 4). Such varying radiation sensitivities have earlier been observed by others too. Thus, as early as 1955, Gregory observed a wide range of sensitivity of X-rays among different strains of peanuts. Later varietal differences in the radiation sensitivity were reported by Brosk (1965 a) on various crops; Ankineedu et al. (1968) in castor, Siddiq (1967) in rice and Sharma et al. (1970) in pea.

Thus radiation has been proved highly effective for Lens and it can be usefully employed for further breeding. However, in case of Linum, it does not appear to be very fruitful for further exploration in breeding work.

OBSERVATION IN R_2 GENERATION

a) Frequency and spectrum of macromutations

Frequency of mutation

The chlorophyll mutations in R_2 generation have been proved to be most dependable indices for evaluating the genetic effects of mutagenic treatments (Gustafsson, 1951). In the present experiments mutation frequencies in both the crops were calculated in terms of (i) percentage of families segregating for mutations and (ii) percentage of mutant plants in R_2 population.

For both the varieties higher dose of gamma rays has been found to be potent, which induced highest chlorophyll mutation frequency (Tables 15 and 16).

Along with chlorophyll mutation frequencies, the rate of morphological mutations also increased in both genera with the increasing intensity of dosage (Tables 13 and 14) and it is quite in conformity with results obtained for other crops (Pavret, 1963 in barley; Blixt, 1964 in pea; Rai and Das, 1975 in linseed). Bozzini and Scarascia Muneozza (1970) in durum wheat found a positive corrèlation between dose and mutation rate which coincides with the results of the present work.

Radiation sensitivity and mutation rate in R_2 generation

In the present course of investigations, it was observed that the mutations induced by radiation was higher in *Lens* in comparison to *Linum*. Thus *Lens* is proved more sensitive to the effect of radiation than *Linum*.

The data concerning R_1 damage as well as mutability revealed that in the two cases Linum is more resistant to the effect of radiation. These findings are in conformity with the results of Sidorova et al.,(1966) and Hasan (1980).

Spectrum of mutations

Chlorophyll mutations

From both the crops, four types of chlorophyll mutations i.e. Albina, Viridis, Xantho-alba Chlorina (Table 17-18) were isolated.

The rase albina mutant occurred with lowest frequency in the present studies (Table 17 and 18). Similar results were reported by Nayar (1969) in Sesamum, Marki and Bianu (1970) in Flax, Hussein et al., (1974) in pea, Meono (1977) in Phaseolus and Tsukuda et al.,(1977) in rice, Hasan (1980) in Sesamum.

Morphological mutations

In the present investigation, it was observed that the induced mutation spectrum was broader in Lens in comparison to Linum. In both the crops mutations affected almost all parts of the plants (Tables 7 & 8) but in *Linum*, which has been found to be comparatively less sensitive to radiation, a narrow mutation spectrum was observed.

It was also found that the spectrum of mutations is dependent upon the material used for study. For example oily seeds resist the effect of radiation while the proteinaceous seeds are susceptible to radiation.

Differential spectrum of viable mutations as observed in the present experiments has been reported by several workers (Kobayashi, 1964; Upadhya and Swaminathan, 1969; Sethi and Gill, 1971) Bandyopadhyay and Bose (1979), compared the effects of different doses of radiations on Pea and found that each of the dose induced particular mutations in a relatively large number, which were produced rarely by other dose. Nilon (1967) has reviewed various reports ~~xon~~ alteration in the mutation spectrum induced by radiation or treatment conditions and has concluded that while different mutagens and treatment procedures may cause some changes in relative proportions of different types of mutations in higher plants, a precise control over the spectrum is yet to be achieved.

<u>Types of induced mutations</u>

In both the crops mutations affecting height, branching, leaf, fruit were isolated. The chimeral structures for various characteristics were also produced in R_2.

Mutants show a specific group of different abnormalities and the group as a whole is transferred from one generation to the next, which may be attributed to one of the following reasons.

i) Single gene is responsible for whole character.

ii) Small portion of a chromosome is altered containing many genes.

iii) Group of closely linked genes mutated.

Beneficial mutants in both the plants leading to high yield and increased number of fruits have been obtained indicating a direct qualitative improvement over the parental types. The characteristics of some mutants is summarised in Tables5, 6, 7, 8, 9, 10, 11 and 12.

Mutagenic effectiveness and efficiency

1) Mutagenic effectiveness

Tables 19 and 20 clearly indicate that mutation rate in higher dose of gamma rays was higher in comparison to lower doses in both the plants. Hence higher dose (30 Kr) has been found comparatively more effective in both cases. Siddiq (1967) reported higher mutagenic effectiveness of NMU than neutrons, EMS and gamma rays. Prasad (1972) working with Triticum durum variety NP-404 observed that NMU is the most effective mutagen as compared to NG, EMS and gamma rays.

In the present work the effectiveness of gamma rays calculated on the basis of R_2 progenies mutated, has been found to be higher with higher doses. The increase in the mutation rate was of the same order as the increase in the mutagenic dose. Gupta and Yashvir (1973) reported that in Foxtail millet the effectiveness generally decreased with increasing dose of mutagens. The present study did not confirm such earlier findings that as the dose increases effectiveness decreases. It rather reveals that effectiveness is in a direct linear correlation with the dose rate.

Mutagenic efficiency

It is evident from tables 19 and 20 that gamma rays are not equally efficient in both the species. Though the rate of mutation induced by different doses of gamma rays were significant but the difference with respect to seedling height was rather negligible. Prasad (1972) compared the efficienty off gamma rays, EMS, NMU and NG at biologically comparable doses in Triticum durum variety NP 404. He observed that NMU was reasonably efficient when used at low concentrations.

These observations provide sufficient evidence to prove that mutagenic effectiveness increases and efficiency generally decreases with the increasing doses of mutagens. It would thus be seen that higher mutagenic effectiveness or efficiency does not reflect the mutation frequency and they cannot be used as indices for maximisation of mutation rate.

Micromutations in R_2 generation

In most of the studies on artificial induction of mutations for crop improvement, usually a greater stress has been laid on the induction of drastic changes of the phenotype brought about by mutational changes of the major genes. On the other hand the genetic control of quantitative traits is exerted through a large number of genes understood to be as minor genes, where each single gene contributes a little bit to the total variability. Although the importance of minor mutations in evolution was stressed by Stubbe and Von Wettestein as early as 1941, only in the last few years, effects of the physical mutagenic agents have been evaluated in relation to "minor" genes which control the quantitative characters (Scossiroli, 1965).

In the past no systematic attempt was made to compare the induced additional variability for quantitative traits through induced mutations in *Lens* and *Linum* by gamma rays. In the present investigation an attempt was made to induce variability for economic characters, like days to flowering, average number of branches per plant, average number of fruits per plant, seed weight of 1000 seeds, seed yield etc. in these important oily (*Linum*) and proteinaceous (*Lens*) seed crop. The results obtained are discussed below.

i) <u>Number of days to flowering</u>

In both the crops with all treatments given, there was an increase in the range and variability with respect to average number of days after which flowering was initiated due to induction of early as well as late flowering both in R_2 (tables 21 and 22) generation. The increase in variability was more towards earliness than the lateness. On the basis of R_2 data no relationship could be established between the dose of the radiation and extent of variability induced in both the cases.

The results of present studies are in conformity with the earlier findings of Kao *et al*., (1960), Brock and Latter (1961). However the late flowering plants were far more numerous than the early flowering ones, which resulted in the shift of curve towards lateness in R_2 generation (Figs. 16 A & B). The results clearly indicate that in *Lens* the direction of micromutational variability is more towards earliness and in *Linum* towards lateness.

Based on the studies of subterranean clover and *Arabidopsis thaliana*, Brock (1965 a, 1966 and 1977) proposed a general hypothesis for the behaviour of induced mutations for quantitative characters. According to this hypothesis "random mutations could be expected to increase the variance and shift the mean away from the direction of previous selection history. The response of an unselected character will depend, not only on its previous selection history, but also on the condition as to whether it is genetically correlated with the selected character". On the contrary Gaul and Aestveit (1966) are of the view that the random mutations may bring about an unidirectional change in the mean value of almost every quantitative character of interest to the plant breeder. They studied the genetic variability for culm length of two dwarf and a tall winter wheat varieties treated with EMS and X-rays. In the treated population, the mean culm length decreased in both the varieties. The frequency of distribution was similar and the variability was increased towards the direction of less mean culm length in both the varieties. However they could not succeed to have a further increase in culm length of the tall varieties to induce additional vitality. From these results they concluded that Brock's hypothesis cannot be universally valid. Thus, the direction of micromutational variability is independent of genotype but associated with vitality.

In the present studies, this question has been re-examined using two genera Lens and Linum differing in their flowering period, as a test material and the results obtained lend support to the hypothesis of Brock (1967). Bansal (1969) also supported Brock's hypothesis on the basis of results obtained with barley and wheat varieties of known selection histories.

The components of variation viz. phenotypic and genotypic co-efficients of variation increased with the treatments in both the genera in R_2 generation. Thus a part of induced variability is genetic which increased the heritability and genetic advance over the control to an appreciable extent in R_2 generation. The estimates of heritability and the genetic advance in R_2 generation were not dependent on dose. A considerable sincrease of genetic advance in the treated populations of both the varieties indicated that there is a great scope for selection of early plants.

The increase in the genotypic variance, heritability and genetic advance for number of days to flowering following treatments of mutagens has been reported by several workers (Papa *et al*, 1961; in soybean; Nayakar, 1976 in Niger). The findings of the present experiments are in agreement with the results of these workers.

Average number of branches per plant

In R_2 generation average number of branches per plant with all doses of gamma rays decreased as compared to control while in *Linum* higher doses (25 and 30 Kr) induced branching. It is evident from the tables 23 and 24 (Fig. 17 A & B) that higher doses of gamma rays suppressed the branch formation on both the crops.

Variance for number of branches also decreased in all the treatments.

In higher doses of gamma ray phenotypic and genotypic variability increased in *Linum* while in *Lens* it increased in lower doses also.

Heritability and genetic advancement also increased in higher doses of gamma rays in both cases with genetic advancement. It indicates that the branching is induced genetically and this mutation is of importance for oil as it increases the yield in next generation.

<u>Number of fruits per plant</u>

In R_2 generation average number of fruits per plant was found to be altered in both the species ~~altered~~ in comparison to controls under all treatments ~~in~~ (Tables 25 and 26). Borojevic (1966) in wheat reported a decrease in number of Kernels per plant in M_2 and an increase in R_3 generation to become either equal or greater than the control. The increase of kernels per plant in R_3 generation ~~was~~ has been attributed to the effect of selection, because completely fertile spikes were taken as parental material of R_3 generation.

The increase in the variability of fruit per plant in both the varieties is based on the increase in the range, variance, phenotypic co-efficient of variation and frequency distribution curve in R_2 (Fig. 18 A & B). The validity of results of the present study is supported by the earlier reports of several workers, who also found an increased variability of fruits per plant (Bhatia et al., 1970 in Pea; Abo-Hegazi, 1973 in lentil; Rao, 1974 in Pigeon pea; Dixit, 1975 in Sesame; Gupta and Gupta, 1977 in Sesame). Thus, it seems quite possible to generate a large amount of genetic variability for fruit bearing intensity in the crop plants by using physical mutagens.

The increase in the genotypic co-efficient of variation in R_2 generation with the treatments given over control in both species revealed that a part of the induced variability is genetic and was transferred to next generation. The enhanced genotypic variation caused an increased heritability and genetic advancement in both the varieties. Similar results have been reported by Borojevic (1966) in wheat.

<u>Weight of the 1000 seeds</u>

In both the varieties average weight of seeds altered under different doses of gamma rays where variability was observed both in upward and downward direction (Tables 27 and 28) in R_2 generation. The increased variability is evidenced in R_2 generations from the increase in range, variance and phenotypic co-efficient of variation as well as from the phenotypic frequency distribution. Many woskers have earlier reported induced variability for seed characters in crop plants (Koo, 1962 in oats; Gaul, 1963 in barley; Rajput, 1974 in Mungbean; Rao et al., 1976 in Triticale; Gupta and Gupta, 1977 in Sesame).

The increase in the genotypic variability in R_2 generation was reflected in the form of increased heritability and genetic advance with all the treatments given except with a few ineffective ones. Such as increase in the genotypic variability, heritability and genetic advance for seed weight in the mutagenised population has also been observed by Koo (1962) in oats and Ibrahim and Sharean (1974) in wheat. Thus, it is possible to select plants with higher seed weight in R_2 generation.

v) <u>Seed yield per plant</u>

The average seed yield per plant decreased in R_2 generations of both genera with all the treatments given. The decrease in average seed yield capacity in R_2 generation was due to a decrease in pollen fertility directly effective on capsule frequency and also due to other detrimental mutations. Scossiroli (1966) also observed a decrease of the seed yield per plant in <u>Triticum durum</u> in M_2 population, which increased in M_3 generation. They considered this change as a recovery effect, and attributed it to the elimination of bad genes after selfing. Similar results were obtained by Gaul and Aastveit (1966) in hexaploid wheat.

The data on range, variance and phenotypic co-efficient of variation revealed that there was a net increase in variability in R_2 generations. The comparison of frequency distribution curve in R_2 (Tables 29 and 30) generation (Fig. 19 A & B) indicated variability was the highest in R_2 generation. The curve shifted more towards higher seed yield per plant in R_2 generation. The increased variability for seed yield per plant has been reported by several workers in different crops (Koo, 1962 in oats; Gaul, 1963 and Bansal, 1969 in barley and Rao *et al.*, 1974 in Triticales).

Although the seed yield per plant with most of the treatments given, was less than the control in R_2 generations, yet the genotypic variability increased with all the treatments due to an increase in the range and overall variance. The increase in the genetic component of variation further enhanced the heritibility and the genetic advance. Several workers have reported an increase in genotypic variability and other genetic parameters in the treated population (Papa *et al.*, 1961 in soybean; Koo, 1962 in oats and Gill *et al.*, 1974 in barley).

From the present study it may also be concluded that the oil present in *Linum* seed protects it from the effect of radiation and therefore radiation is not an important tool for mutation breeding in oily crops. While proteinaceous crops like *Lens* is more affected by radiation and is an important tool for mutation studies in this crop.

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SUMMARY

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SUMMARY

Presoaked seeds of two plants *Lens culinaris* variety - JLS-3 and *Linum usitattissimum* variety - NP (RR)-5 were irradiated with 5, 10, 15, 20, 25 and 30 Kr doses of gamma rays from Co^{60} source and the effect of irradiation was studied in R_1 generation on seed germination, growth-rate, mortality and survival of seedlings, pollen fertility, seed yield and morphological abnormalities, and in R_2 generation, on mutagenic sensitivity of the two plants, frequency and spectrum of chlorophyll, viable macro and micro mutations, mutagenic effectiveness and efficiency, micromutations with respect to quantitative characters. R_2 and R_3 generations were also raised to study behaviour of quantitative characters of economic importance like days to flowering, number of branches per plant, number of fruits per plant, weight of 1000 seeds and seed yield. Some of the data was statistically analysed which includes computation of mean, overall variance, phenotypic and genotypic coefficient of variation, heritability and genetic advance (expressed as percentage of mean).

Slight shift in the initiation of germination of the irradiated seeds was observed both in *Lens* and *Linum* due to the marked decline in their germination percentage with increasing dosage of irradiation, except 5 and 10 Kr doses in *Linum*, where the germination percentage was found equal to that of control. A comparison of the data revealed that in respect to seed germination, *Lens* is more sensitive than *Linum*.

Growth rate of both root and shoot of the progenies raised from irradiated seeds has been found to be markedly hampered. Growth rate in both, more or less uniformly followed a linear inverse correlation with the dose rate, which was particularly more pronounced in *Lens* in comparison to *Linum*. Interpretations in this respect given by others have been reviewed. Data on growth has confirmed the reports of earlier workers.

Data on seedling survival indicated an inverse correlation with the dosage of gamma rays. Seedlings of *Lens* showed slightly higher mortality than those of *Linum*. In this respect also *Lens* has been found more sensitive to gamma radiation than *Linum*.

Variations in shape and size of leaves, chlorophyll chimeras and morphological abnormalities of other plant parts were observed in *Lens* and their abundance has been found to be directly proportional to the dose rate, while in *Linum* lower doses 5, 10 and 15 Kr doses did not produce any type of chlorophyll chimeras and morphological abnormalities.

Pollen fertility has also been found to be inversely correlated with the dose rate. *Lens* has been found to be more highly affected in this regard. Under 5 Kr dose in *Linum* pollen sterility was not observed.

Seed output in both *Lens* and *Linum* has been observed markedly reduced under the influence of gamma irradiation and it has also shown an inverse linear correlation with the dose rate. Here also in *Linum* lower doses of gamma rays were ~~proud~~ found comparatively ineffective but higher doses produced thin papery seeds. In *Lens*, size of seeds was much reduced with higher doses of gamma rays, with decreased weight and volume. In this respect, *Lens* is more highly effected than *Linum*.

Significant difference in the mutagenic sensitivity was observed between Lens and Linum. Linum was found to be more resistant towards various irradiation doses than Lens. Hence Linum is radio-resistant while the Lens is radio-sensitive. Higher doses of radiation were more effective inscomparison to lower doses in Lens, while in Linum lower doses are ineffective.

The frequency of chlorophyll and viable mutations was higher in Lens than in Linum even with the same doses. It was also found that chlorophyll and viable mutation frequency was directly correlated with dose rate. Lens which is highly radiosensitive yielded maximum mutations. The results suggested that, in general, the germ plasm with comparative higher mutagenic sensitivity is also more mutable than the less sensitive germplasm.

The spectrum and the relative proportion of different chlorophyll mutations were more or less similar in both Lens and Linum. Various types of chlorophyll mutations obtained were Albina, Chlorina, xantha-alba and Viridis. But in Linum there was no chlorophyll mutations in lower doses. Different morphological mutations were also recorded in both varieties. The mutations affected almost all parts of the plants. The spectrum of morphological mutations were narrow in Linum and broader in Lens. The mutation spectrum has been found to be dependent upon the dose of radiation. In general, the effectiveness of mutagens was found increased and efficiency decreased with the increasing dose levels. Neverthless highest mutation rates were obtained at higher doses of radiation treatment.

When the experimental material was analysed for induced polygenic variability, it was found that in the mutagen treated populations the mean value of some of the characters decreased in R_2 but subsequently increased in R_3 generation. The depression in the average values of these characters in R_2 is probably the outcome of the residual damaging effect of mutagenic treatments and the improvement in R_3 is due to the recovery from such a damage.

The range, overall variance, phenotypic co-efficient of variation and frequency distribution curves revealed that as a result of radiation treatments, the variability increased for all the characters in R_2 and R_3 generations. For most of the characters induced variability remained almost unaltered in R_2 and R_3 generations.

There was no apparent relationship between the dose of radiation and the extent of the variability induced. It has been inferred that selection should be made preferably from R_3 generation where the genetic variability is clearly manifested and some genetic stability is reached.

The increase in the genotypic coefficient of variation with the various treatments for all the characters in R_2 suggested that a part of variability recorded was genotypic, which increased the heritability and genetic advance (expressed as percentage of mean). In R_3 generation, the genotypic co-efficient of variation, heritability and genetic advance remained unchanged for almost all the characters.

The results indicated an additional variability when the treated populations were advanced in R_2 and R_3 generations. It thus became evident that there is sufficient scope for selection of beneficial morphological macro mutation from R_3 progeny of the treated population. But in case of ~~see~~ seed yield the variability was almost equal in R_2 and R_3 generations. This fact provides scope for selection of plants with seed yield from R_2 as well as R_3 generations with equal chances.

It has also been found that the oil present in the seed of _Linum_ protects them from the effect of radiation in all biological parameters hence oily seed of _Linum_ are radio-resistant. But the seeds of _Lens_ which contain proteins are vulnerable to radiation as revealed by the frequency and spectrum of different micro and macro mutations observed in the present study.

It has been concluded that gamma ray treatment may not be useful for the improvement of micro and macro beneficial mutants in Linseed

(*Linum usitattissimum*) and is less effective, while it has been also concluded that gamma irradiation is useful tool for the improvement of beneficial mutants in Lentil (*Lens culinaris*) and is effective and can be used for improvement of this crop plant.

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